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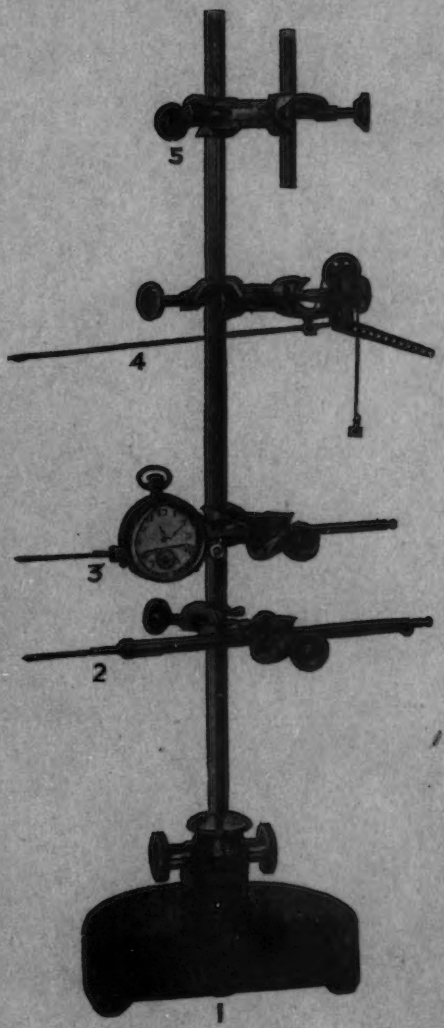

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ON THE VENTRICULAR ELECTROGRAM OF THE FROG'S HEART OBTAINED THROUGH INDIRECT LEADS

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From the Pathological Laboratory of the University of Amsterdam

Received for publication September 29, 1925

In a previous publication I (1925) demonstrated that Lewis has not proved that the excitation wave is conducted at a ten times quicker rate through Purkinjé's network than through the muscle of the ventricular wall. The results obtained by Lewis were largely due to experimental conditions. Furthermore I showed that his interpretation of the origin of the R- and S-deflection in the heart of cold-blooded animals is wrong, that it is so at least for the direct lead. Lewis (1922) thinks that the R-deflection is to be ascribed to the fact that initially the main direction of the excitation wave through the ventricle is from base to apex. He believes that the excitation wave first reaches the center of the surface of the ventricle, and that it propagates from this point toward the apex, and toward the base as well. According to him the main direction from base to apex is predominating at the beginning and hereby he supposes the R-deflection to be generated. The negative S-deflection he believes to arise because "the basal segments of muscle are the last supplied." In this way, according to Lewis, apical activity might give a deflection upwards (i.e., in the same direction as the P-deflection), and basal activity a deflection downwards. He tried to find evidence for this conception in the fact that in an electrocardiogram of a toad's heart the bulbus arteriosus gives a downward deflection (opposite to the direction of the R-deflection). Now in a previous paper I have demonstrated that, anyway for the direct lead, this hypothesis is wrong. As Lewis declares that it is exactly to the indirect leads that his hypothesis is applicable, I have continued my experiments with indirect leads.

I have experimented mainly on rana esculenta and also on rana temporaria. For the registration of the action-currents I employed the leading-off cavity of the mouth-abdomen so that its direction coincided

with the long axis of the heart. After the frog had been dehematised and the spinal cord and the brain had been destroyed, first an electrocardiogram was taken of the intact heart. For this purpose the heart was laid bare, the pericardium was cut open, so that for the rest the condition was quite similar to that before the following registration, except that a transverse incision was made in the ventricle. When this transverse incision was made, the action-currents of the heart were again recorded ceteris paribus.

Just as in my previous investigation, four series of experiments were conducted:

1. In the first series a transverse incision was made in the ventricular base. The upper line of figure 1 shows the E.C.G. before the incision. Here we observe a P-, an R- and a T-deflection, also a small C-deflection. The latter is caused by the bulbus arteriosus; it has the same direction as the P-deflection. The second line shows the curves that appeared after

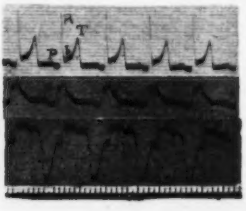

Fig. 1

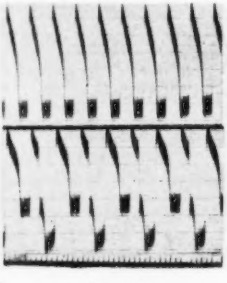

Fig. 2

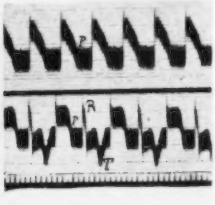

Fig. 3

the incision in the base. Above the incision the base now contracts after each auricular systole, but the other part of the ventricle remains quiet. Now each P-deflection is followed by a slow upward deflection similar to the basal component of the ventricular electrogram. On examination it appeared that at this moment only the portion of the base above the incision contracted after each auricular systole. After about five minutes the conduction along the muscle bridge was restored and after each contraction of the base the remaining part of the ventricle also began to contract. The electrogram that now came forth has been reproduced in the bottom line of figure 1. We now see that after the basal component the curve of the electrogram suddenly deflects downward. So the moment when excitation wave is propagated from the base along the muscle-bridge towards and through the apex there appears a downward deflection. It is beyond doubt, therefore, that the main direction of the excitation wave is now *from base to apex*. We see then that in these electrograms

the initial upward deflection is caused by basal activity, while the next following downward deflection is caused by apical activity.

2. Figure 2 shows the electrograms of another frog's heart, after a transverse incision in the middle of the ventricle. In the upper line we see the electrogram of the base above the incision. During this registration only the base contracted after each auricular systole. Five minutes later every second basal contraction is followed by an apical contraction below the incision. In the electrograms we see, at the moment when the excitation wave passes along the muscle-bridge, an abrupt downward deflection. In this series also there originated an upward deflection through activity of the base and a downward one through apical activity.

3. In the third series an incision was made in the ventricular apex. In figure 3 we see the curves that appeared after the incision. In the upper line we see the electrograms recorded directly after the incision. The portion of the ventricle above the incision contracted only as the registration was proceeding. A couple of minutes later every second basal contraction was succeeded by an apical one. Under the influence of apex-activity the initial upward deflection was reduced to zero and at the end a negative T-deflection arose.

4. The experiments of this series, in which an incision was made in the auriculo-ventricular groove, without injuring the ventricle, yielded results similar to those of the previous investigation with the direct lead. Fundamentally the ventricular electrogram was not modified by it.

Theoretical considerations. The present investigation goes to show that also with indirect leading-off, the direction of which coincides with the long axis of the heart, the ventricular electrogram is the interference product of the basal and the apical negativity. It has appeared, moreover, that also with the indirect lead the deflections of the ventricular electrogram are not brought about by the direction in which the excitation wave is propagated through the ventricle in the sense suggested by Lewis. This is evident from the fact that in the first three series of experiments in which a transverse incision had been performed in the ventricle, the excitation wave is propagated, after passing along the narrow muscle-bridge, through the apical portion in the direction from the base towards the apex. According to the hypothesis of Lewis there should appear an upward deflection in the electrogram, whereas in reality there appears a downward one. *Ergo*, Lewis' hypothesis cannot be right.

In my previous paper I was obliged to limit my conclusions to the direct lead. It now appears that all of them apply in every way to the indirect lead also. In order to save space I shall not repeat them here and will only refer to my preceding communication.

I feel, however, prompted to add a few more remarks: As to the direc-

tion of the excitation wave through the ventricle, Clement (1912) came, by means of local leading-off with differential electrodes, to a conclusion which was the very opposite of Lewis'. Whereas Lewis believes that the excitation wave is propagated through the base in the apex-base direction, and through the apex from base to apex, Clement (1912) adopted the opposite view. Clement concluded from his experiments with local leading-off that, in the frog's heart, the excitation wave is propagated through the base from the auriculo-ventricular groove toward the apex, and through the apex in the direction apex-base. It is evident from my experiments of the previous and the present paper that, as regards the base, it is a fact that the excitation wave is propagated in the base-apex direction. My experiments do not indicate in which direction the excitation wave proceeds through the apex in the intact ventricle of the frog's heart. For my interpretation of the ventricular electrogram the direction of the excitation wave through the apex is of no consequence. This interpretation is quite in harmony with Clement's conception of the direction in which the excitation wave propagates through the ventricle.

Lewis attaches great importance to the direction of the bulbus-deflection in an electrogram from a toad recorded by him. From this it might be inferred that the bulbus always yields an electrogram deflection of a direction opposite to that of the P-deflection. For this reason I have reëxamined my previously registered electrograms of the frog's heart and found that with direct lead the bulbus deflection is often directed upwards, sometimes it is biphasic, and the bulbus can also yield a downward deflection in direct lead. I recorded an upward bulbus deflection in Pflüger's Arch., cxcii, p. 206 (figure 10) and Arch. Néerl. des sciences exactes et naturelles Série 3B, Tome 2, p. 555 (1915) (fig. 6). A bisphasic bulbus-deflection in Zeitschr. f. Biologie, lxv, p. 434, (1915) (fig. 4). A downward deflection in Pflüger's Arch., clxxxvii, p. 224, (1921) (fig. 20).

Figure 1 of the present paper gives an instance of an upward bulbus-deflection with an *indirect* lead. For the present I think it better to abstain from drawing any further conclusions, for the ventricular electrogram, from the direction of the bulbus deflection. I can say in addition, that the bulbus deflection in Lewis' figure occurs at a much later phase of the ventricular electrogram than I invariably found it in the frog's heart.

SUMMARY

1. In the above investigation it has been proved that in the frog's heart the contraction of the base gives, under indirect leading-off, a deflection of the same direction as the P-deflection in the auricles, and the contraction of the apex a deflection opposed to the direction of the P-deflection.

2. The direction of the deflections of the frog's ventricular electrogram is, under indirect lead, determined also by the various conditions of one of the leading-off electrodes or of both.

3. The direction of the deflections R. and S. of the ventricular electrogram of the frog's heart is, under indirect leading-off not determined by the direction of the contraction waves through the ventricle in the sense suggested by Lewis.

4. Under direct leading-off the electrogram deflection of the bulbus arteriosus of the frog's heart can be directed upwards or downwards or can be biphasic. Under indirect leading-off it may be directed upwards.

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A METHOD FOR SEPARATING THE AIR BREATHED BY THE RIGHT AND LEFT LUNGS, TOGETHER WITH THE EFFECT OF PULMONARY CIRCULATORY CHANGES ON THIS DIVIDED BREATHING

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There are, in general, two methods of approach to the study of the relations between the volumes of blood and air in the lungs. The first of these is through a consideration of the effects of changing volumes and pressures of ventilation on the pulmonary circulation. The fact that these changes occur during every inspiration and expiration has made their effects easily available for study with the development of satisfactory methods of measuring blood flow and pressure in the lesser circulation. The results of these studies have been reviewed by Tigerstedt (1) and by Wiggers (2). The most extensive experimental work has been done by Cloetta (3), who concludes that the conditions are most favorable for pulmonary blood flow during the first part of inspiration, presumably because of a mechanical dilatation of the intrapulmonary vessels. The conditions favoring blood flow are at their worst at the end of inspiration through compression of the vessels, but become more favorable again during expiration.

The study of the relationships between intrapulmonic blood and air by the second method of experimentally altering the blood flow and observing the effect on ventilation, has been more difficult, but has been approached in several different ways. Romanoff (4) has summarized the preceding experimental data on the effect of distention of the pulmonary capillaries on the alveolar air-space as found by perfusing the dead organ. With additional studies of his own, he concludes that distention of the capillaries causes an increase in the intrapulmonary air-space as long as the lung meets no resistance to its enlargement (as with an open chest); but with a limitation to expansion (as with a closed chest) capillary distention is attended by a diminution of the air-space.

Yates (5), reasoning from the clinical phenomena attending compensatory emphysema, and from unpublished experimental work, postulates

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an "air-cell-capillary gear" mechanism through which capillary distention brings about an increase in the volume of the corresponding alveolar air-spaces. This mechanism serves to maintain a constant ratio between the volume of intrapulmonic blood and the air available for respiratory exchange.

Graham (6), in refutation of Yates' theories, has attempted to demonstrate by histological methods the encroachment of distended capillaries on the alveolar spaces.

Drinker, Peabody and Blumgart (7) showed that less air entered the lungs when a congestion of the pulmonary capillaries was produced by compression of the pulmonary veins in an animal being artificially ventilated at a constant pressure. These results were interpreted as having an explanatory bearing on the reduction in vital capacity known to accompany cardiac decompensation.

Head (8), in effecting varying degrees of distention of one lung, employed a cannula which was inserted into the primary bronchus through the trachea. The cannula was provided with an India rubber collar which was distended by the injection of glycerine through a separate tube, in order to produce an air-tight connection in the bronchus. This method was quite suitable for his work with artificial ventilation, but would be attended by too great an encroachment on the air-way to be employed in a naturally breathing animal.

Mathieu and Hermann (9) state that they have subdivided the trachea of a dog by a longitudinal partition and collected the expired air in two spirometers, but do not explain their technique.

The following method was designed to determine the relation between varying conditions of the pulmonary circulation and the air-space in the lungs of an animal breathing with natural respiration. Briefly, it consists in collecting the air moved by each lung in separate spirometers, and at the same time altering the conditions in the pulmonary circulation on one side by clamping either main branch of the pulmonary artery, or either group of pulmonary veins.

METHOD. A cat is completely anesthetized by an intravenous injection of sodium barbital (5 per cent solution), and temporarily placed on artificial respiration while an elliptical section including the sternum is cut from the anterior aspect of the thorax. The left primary bronchus is isolated by blunt dissection in the posterior mediastinum, and a ligature passed around it. The approach to the bronchus is made just below the arch of the aorta, and care must be taken not to injure the phrenic and vagus nerves which are retracted anteriorly. One of a pair of brass tubes which up to this point have been utilized in maintaining the intratracheal positive pressure ventilation, is then guided into the left bronchus and anchored in place by the ligature which lies just below the bifurcation of the trachea.

The ventilation of the right lung is maintained through the other tube of the same length and caliber lying in the trachea. These tubes are especially designed to avoid any material reduction in the cross-section area of the available air-way, having a very thin wall and the largest diameter which is possible for the trachea to accommodate. An air-tight closure of the trachea about the tubes is made in the neck by packing with bits of muscle and applying a tight ligature. In a satisfactory preparation all lobes of both lungs expand and deflate equally with the same degree of positive pressure ventilation.

The pericardium is then opened by longitudinal incision and its edges sutured to the margins of the window cut in the chest after the method described by Drinker (10) and now routinely used in this laboratory. This forms an air-tight closure of the pleural cavities, and after the residual air has been removed by aspiration, the artificial ventilation is discontinued and the animal resumes natural breathing. The presence of any air in the chest from a faulty preparation is at once evident by direct inspection of the pleural cavities through the transparent pericardium. A pair of Tissot valves is placed on each tube so that the expired air from the two lungs can be delivered to separate spirometers (fig. 1).

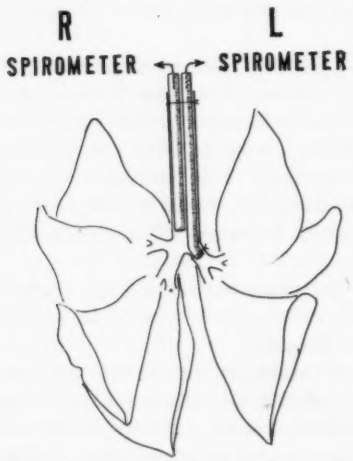

Fig. 1. Diagram showing the position of the tubes employed for the separation of the air breathed by the two lungs.

By blunt dissection from within the pericardium, ligatures are then passed around either the right or the left branch of the pulmonary artery, or the right or left group of pulmonary veins. By the use of a foot clamp with a screw adjuster, (Drinker, Peabody and Blumgart (7)), varying degrees of compression up to complete occusion may be effected by traction on the ends of the ligature. The pressure in the systemic circulation is continuously recorded by a femoral artery cannula and a mercury manometer. At the conclusion of the experiment the animals are killed in a uniform manner by section of the pulmonary artery.

To summarize: In such a preparation we are dealing with a cat breathing naturally with the chest closed. Each lung moves its air through a separate air-way, and the expired air from the lungs may be collected in different spirometers and the minute-volumes ascertained. The

pulmonary circulation may be altered by temporary occlusion of either branch of the pulmonary artery, or either set of pulmonary veins; and under the varied conditions so produced the volume of air moved by each lung may be recorded.

The minute-volumes of tidal air collected in the spirometers bear no significance as absolute values, or as a direct index to the alveolar air-space, to which they must bear varying relationships under respiratory stimuli of different intensities. The findings which may be considered significant are the changes in the ratio between the volume of air moved by one lung and that moved by the other, which are brought about by simultaneously subjecting the two lungs to different circulatory conditions.

EXPERIMENTS. The following experiments are presented graphically rather than by complete protocol, and are illustrative of the results obtained with the preparation described above.

1. Control period followed by right pulmonary artery occlusion. (*Fig. 2.*) The first portion of this experiment is devoted to a control period which shows the degree of constancy maintained in a satisfactory preparation not only in actual minute volumes of tidal air, but more particularly in the ratio between the volumes of air moved by the two lungs. The minute-volumes are charted in cubic centimeters by vertical columns. The ratio between the volumes moved by the two lungs is shown by charting the percentage of the total ventilation which the same lung (in this case the right) moves throughout the experiment. The systemic blood pressure is charted in millimeters of mercury.

At the point indicated by the arrow the right pulmonary artery was occluded. The animal immediately responded by an increase in both rate and depth of respiration, raising the total tidal air from 903 cc. to 1317 cc. per minute. During the short period while the occlusion was maintained, this increase in minute-volume was shared proportionately by the two lungs.

2. Occlusion of the right pulmonary artery with release followed by occlusion of the right bronchus. (*Fig. 3.*) The right branch of the pulmonary artery was clamped as in the preceding experiment and two air collections taken. It was then released, and when equilibrium was restored the air-way to the right lung was occluded by placing a clamp on the rubber connection at the end of the brass tube leading to the right lung. The percentage of the total volume which the right lung was moving remained almost constant while the artery to that side was clamped, but of course fell to zero when the bronchus was clamped.

During both procedures the left lung remained the only effective unit of ventilation, and it is to be noted that it showed an almost identical increase in its minute-volume in the two instances. The clamping of the bronchus was attended by a high degree of respiratory difficulty, and was too drastic

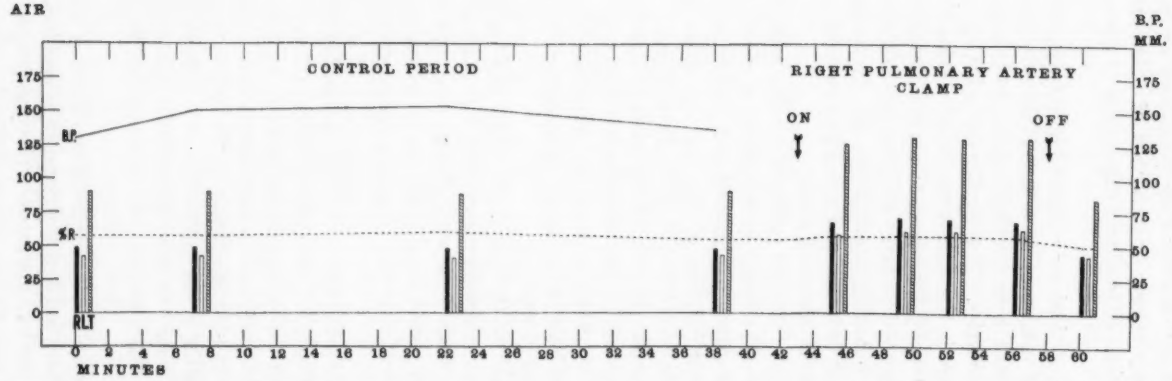

Fig. 2. Experiment 1. Solid line, systemic blood pressure; broken line, percentage of total ventilation moved by the right lung; solid columns, cubic centimeters of air moved by the right lung per minute; open columns, cubic centimeters of air moved by the left lung per minute; shaded columns, total ventilation in cubic centimeters per minute. Reading from left to right the two arrows indicate the points of occlusion and release of the right pulmonary artery. Ordinates, millimeters of mercury; percentage; and, multiplied by 10, cubic centimeters of air. Abscissa, time in minutes.

a procedure to be maintained for any period of time. It was followed by a disturbance in the ratio between the minute-volumes of the two lungs from which the animal had not fully recovered at the termination of the experiment.

This incident is worthy of note, not because we are able to directly interpret its significance, but because it illustrates the delicate and complex mechanics involved in the animal preparation we are using. The cutting away of the sternum, the placing of the ligature about the bronchus with the possible attendant trauma to the vagal plexus, and the insertion of the artificial air-ways are all procedures which are capable of profoundly disturbing the mechanical conditions of respiration. If the preparations were always mechanically perfect in reproducing the natural breathing of the animal, the right lung would always move more air than the left

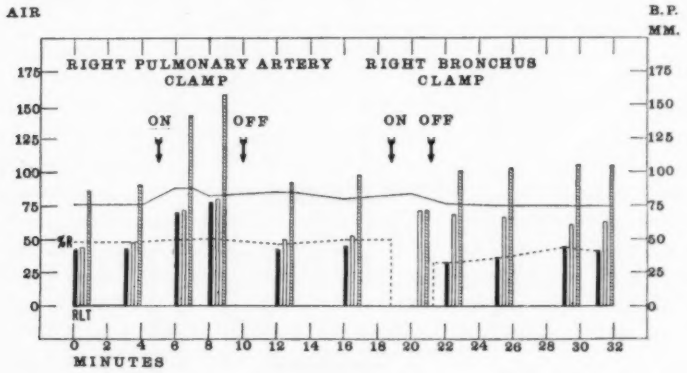

Fig. 3. Experiment 2. General arrangement of chart identical with figure 2. Experimental procedures described in text.

because of its larger volume. As a matter of fact, it does so in a large majority of preparations, but at times in an animal prepared with the same care, the left lung will be found moving more air than the right. Each preparation must be considered as presenting some characteristics peculiar to itself, and must be studied for control before experimental changes are produced.

3. Prolonged occlusion of the right pulmonary artery. (*Fig. 4.*) The right branch of the pulmonary artery was clamped as before at the point indicated by the arrow. The usual increase in minute-volume was at once evidenced, at first being shared proportionately by the two lungs. As the occlusion was continued, the left lung showed a slow but steady increase in its minute-volume, actually from 37 to 47 per cent of the total ventilation. At autopsy the lungs were apparently normal in relative size

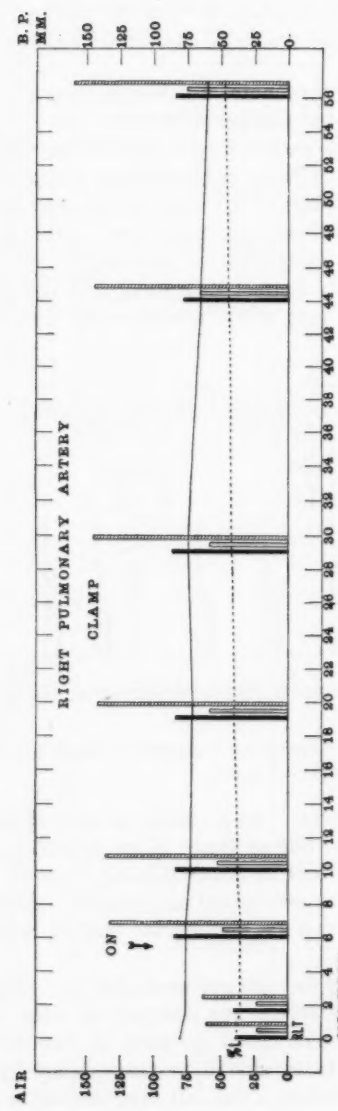

Fig. 4. Experiment 3. General arrangement of chart as before except that the broken line represents the percentage of the total ventilation moved by the left lung.

and in consistency. The right lung was darker in color and appeared to contain more blood on section.

SUMMARY OF THE EFFECTS OF PULMONARY ARTERY OCCLUSION. In attempting to draw conclusions from the above experiments in terms of the effect of distention of the capilaries on the air-space, any assumptions regarding the pressure in the capillaries of the affected lung must be approached with caution. Underhill (11) has found after ligation of the left pulmonary artery and subsequent closure of the chest with resumption of natural breathing, that the left lung usually contains more blood than the right. This blood he believes comes from the bronchial arteries and stagnates in the capillaries. His observation on the amount of blood in the affected lung is confirmed by the autopsy findings noted above. If a certain amount of blood does find its way into the lung after the pulmonary artery is occluded, either by the channels suggested by Underhill, or by anastomoses between the pumonary and general circulation which have been observed in this laboratory (Drinker, et al (12)), the degree of capillary distention is difficult to estimate.

On the other hand, direct measurements of the pressure in the pulmonary artery after occlusion of one of its branches are available. Haggart and Walker (13) report that clamping the left branch of the pulmonary artery causes an immediate rise in pulmonary pressure which averages about 29 per cent. A rise of 40 per cent is reported by Underhill following a similar procedure in an artificially ventilated animal. The capillaries in the lung with the unoccluded artery are, therefore, subjected to an increased pressure, whereas those of the affected lung are almost as certainly subjected to diminished pressure, although to what degree we are unable to state for the reasons just cited.

In summarizing the effects of the distention of the pulmonary capillaries produced by the occlusion of one branch of the pulmonary artery, we may say that we have observed no significant immediate change in the relative volume of air moved by the two lungs, and consequently can assume no alteration in the alveolar air-space. In certain instances, at the end of an hour the lung with the greater degree of capillary distention moves relatively more air, a phenomenon which could be interpreted as indicating an increase in its alveolar air-space.

4. Occlusion of the left pulmonary veins. (*Fig. 5.*) Three control collections consistently showed the minute-volume of the left lung to be less than that of the right in the average ratio of 45 per cent of the total ventilation. In the first collection after the left pulmonary veins were clamped the left lung moved more air than the right, 57 per cent of the total. This increase in the relative amount of air moved by the left lung continued steadily up to the time when the clamp was released, thirty minutes after its application. At this time the left lung was moving 75 per cent of the total minute-volume respiration. Release of the clamp was followed by

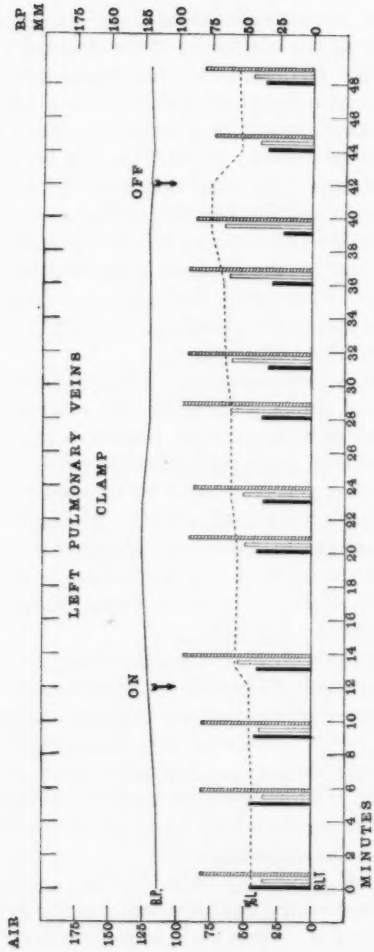

Fig. 5. Experiment 4. General arrangement of chart as in figure 4; arrows indicate points of occlusion and release of the left pulmonary veins.

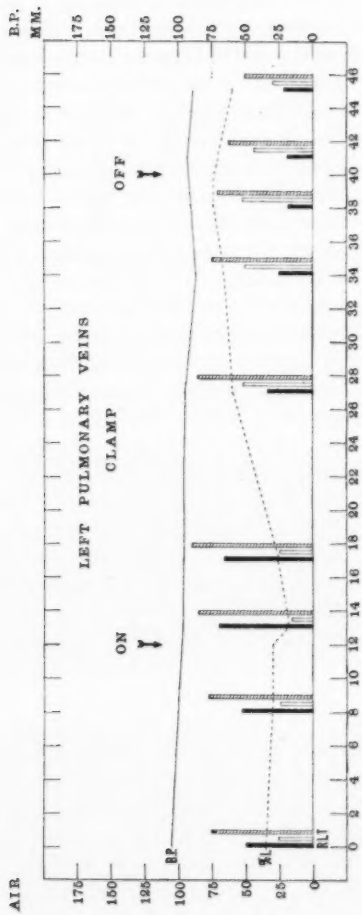

Fig. 6. Experiment 5. General arrangement of chart identical with figure 5.

an immediate drop to 53 per cent. At autopsy the lungs were similar in texture and showed no gross evidence of edema.

5. Occlusion of the left pulmonary veins. (*Fig. 6.*) Starting with the left lung which was moving 33 per cent (average of two control collections) of the total minute-volume ventilation, clamping of the left pulmonary veins was followed by a decrease to 18 per cent. This decrease was very transient, however, and at the end of fifteen minutes the percentage moved by the left lung had increased to 60 and subsequently to 74 per cent. At the time of the release of the clamp the animal was apparently in excellent condition, although a steady fall in the total ventilation during the last half of the experiment is suggestive of a failing respiratory center. After the release of the clamp the respirations rapidly became more shallow, and the animal died with a terminal cardiac dilatation. At autopsy the left lung was very congested, the cut surface appearing bright red in contrast to the pale, normal right lung. On section, frothy fluid exuded from the alveoli, and the bronchial tree was full of frothy fluid.

It seems probable that the death in this animal was due to the rapid onset of pulmonary edema in the left lung following the reëstablishment of its circulation. The autopsy findings make it extremely unlikely that any respiratory exchange could have taken place in this lung after the release of the clamp; and the opening of the circulation through it only served to greatly increase the anoxemia to a fatal point.

6. Occlusion of the right pulmonary veins. (*Fig. 7.*) The clamping of the right pulmonary veins was found to be a much more drastic procedure than the clamping of the left, and it was found necessary to tighten the ligature very slowly to avoid cardiac failure from the sudden drop in systemic blood pressure (fig. 8).

Following the clamping of the right pulmonary veins the animal exhibited a much higher degree of hyperpnea than with the corresponding procedure on the left. The percentage of the total ventilation which was being moved by the lung rose from 45 to 49 per cent and then steadily fell to 40 per cent at the time of the release of the clamp. A further drop to 33 per cent was then recorded. Just before the release of the veins, a difference in the color of the two lungs was noticeable when they were viewed through the transparent pericardium. The right lung appeared bright red in contrast to the somewhat dusky hue of the left. At autopsy the right lung was found to contain more blood than the left, and presented what appeared to be a few mottled patches of edema.

SUMMARY OF THE EFFECTS OF PULMONARY VEIN OCCLUSION. The results of pulmonary vein occlusion have been quite striking, and it is along this line that further data are being obtained at the present time. The experiments here recorded show that with the passive congestion which accompanies the clamping of the left pulmonary veins, the left lung shows a marked increase in its relative ventilation. In one instance this was from

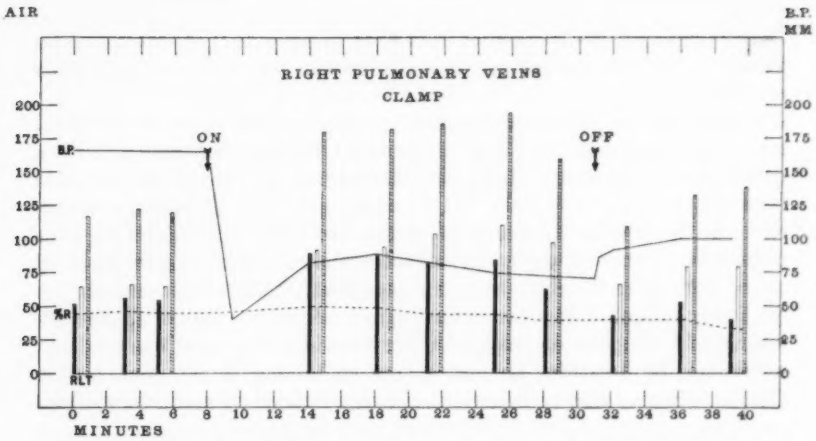

Fig. 7. Experiment 6. General arrangement of chart as before, with the broken line representing the percentage of the total ventilation moved by the right lung. Experimental procedures described in text.

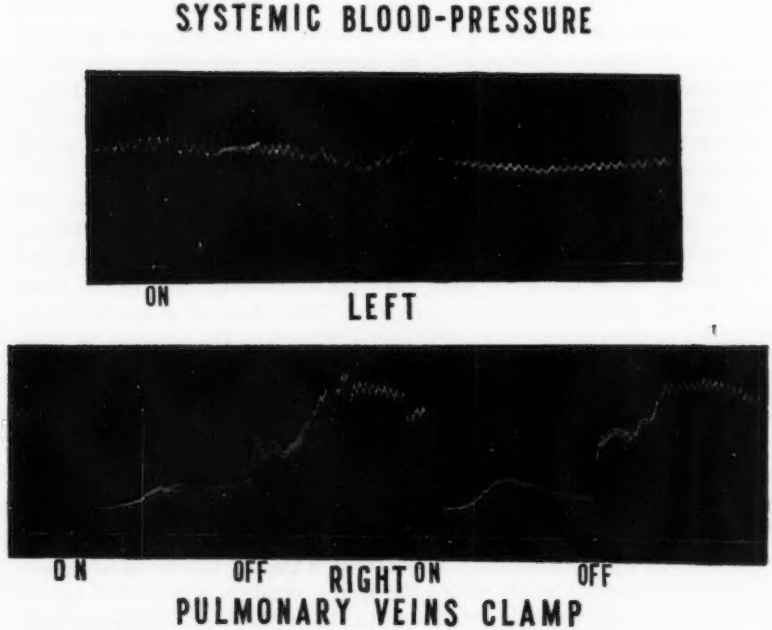

Fig. 8. Tracing of the systemic blood pressure showing the effects of the occlusion of the right pulmonary veins as contrasted with the usual effect observed when the veins from the left lung are occluded.

33 to 74 per cent, and in another from 45 to 75 per cent of the total minute-volume. In both experiments this change was reversed on releasing the clamp.

This effect has not been obtained by clamping the veins of the right lung. In the experiment cited a transient increase was followed by a definite and increasing diminution. The significance of this difference in behavior is not entirely clear, but we would point out concomitant differences in the degree of hyperpnea in the two cases. With the vigorous respiratory exertions which the animal exhibited following the clamping of the veins from the right lung, it is possible that the air-spaces were immediately expanded to a maximum degree. A similar lack of change following vein compression has been observed when an animal was rendered hyperpneic by breathing an atmosphere containing 5 per cent carbon dioxide. It may also be a factor in the experiments on pulmonary artery clamping.

Discussion. Any consideration of the effect of capillary distention on alveolar air-space must start with the axiom that any increase in the total volume of blood in a closed thoracic cavity must be attended by the displacement of a corresponding volume of air—beyond the certain relatively small limits by which the total volume of the thorax for a given phase of respiration may be increased by an abnormal action of the respiratory musculature. This is the fact demonstrated on the dead lung by Romanoff, and in the living animal by Drinker, Peabody and Blumgart. But what is true for the chest as a whole, does not of necessity apply locally to a portion of the lung, or even to one entire lung.

A portion of lung tissue may expand with an increased volume of both blood and air by encroaching upon adjacent areas of lung. Or with a flexible mediastinum, the volume of an entire lung may become augmented at the expense of the other side. The gravitation of blood to the lower lobes which presumably attends the erect posture does not mean that there will be a corresponding volumetric encroachment on the air-space in these regions; on the contrary, it is a familiar fluoroscopic observation that the bases are normally more radiant than the apices, and that the apical regions only "light up" with cough or a forced inspiration.

The experimental data recorded above are, therefore, not contradictory to the previous experimental work from this laboratory, but serve to correlate it with other facts and theories which were apparently conflicting. They do this by showing that distention of the capillaries in a given portion of the lung may be attended by an increase in the corresponding air-space. A possible exception exists under the special conditions promoted by extreme hyperpnea which in itself appears to produce a maximal enlargement of the air-space. The congestion of the capillaries under such conditions would tend to be less (note the experiments of Drinker and Agassiz, (14) in which different types of artificial ventilation were employed), and

that which does occur might theoretically be expected to actually encroach upon the air-space.

This conception of the relationship between the volumes of blood and air in the lungs has, we believe, an explanatory bearing on the mechanism of compensatory emphysema, a condition which has commonly been considered as the result of the mechanical effect of an increased negative pleural pressure on the air cells.

It has been a great privilege to have the critical oversight of Dr. Cecil K. Drinker both in the details of technique and in the consideration and presentation of the results of these experiments.

SUMMARY

In a study of the effect of changes in the pulmonary circulation on the tidal air, a naturally breathing cat was prepared so that each lung moved its air through a separate air-way and the expired air from the two lungs was collected in different spirometers. The pulmonary circulation was altered by temporary occlusion of either branch of the pulmonary artery, or either set of pulmonary veins; and under the varied conditions so produced the volume of air moved by each lung was recorded.

No change of significance was found in the relative volume of air moved by the two lungs after occlusion of one branch of the pulmonary artery. Following the occlusion of the left pulmonary veins, the left lung showed a marked increase in its relative ventilation, in one instance from 45 to 75 per cent, and in another from 33 to 74 per cent of the total minute-volume.

If distention of the capillaries in a portion of the lung is attended by this increase in the corresponding alveolar air-space, such an increase in volume may be made possible by encroachment on adjacent areas of lung. The mechanism involved in this relationship may be an active force in the production of compensatory emphysema.

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THE EFFECT OF FEEDING THYROID EXTRACT ON GASTRIC SECRETION

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Because of the suggestive results obtained by Hardt (1916) in this laboratory, Doctor Carlson suggested that a more complete study be made to test the effect of prolonged thyroid feeding on the amount of secretion and composition of gastric juice. Hardt, by feeding extensive amounts of desiccated thyroid to dogs for two weeks observed a depression in the quantity and quality of the gastric juice during the first hour after feeding.

So far as can be found, no other literature has been published dealing with the experimental results of feeding desiccated thyroid to determine its effect on gastric secretion. However, some interesting clinical observations have been reported in thyroid disorders. Badylkes (1924, 1925) found that large doses of thyroid causes an increase in some subjects. Lockwood (1925), observing patients, found a diminished gastric secretion in both hyper- and hypothyroidism. Johnson (1919), in his studies of causes of hyperthyroidism in soldiers, speaks of the disordered activity of the alimentary canal and complaints of indigestion in several cases, but reports no tests made on the gastric contents. McCaskey (1925), in his study of hyperthyroid cases, states that appetite in these patients is voracious. He discussed several cases with gastric symptoms in which subacidity was found and one with severe gastric indigestion and complete achlorhydria. Dilute hydrochloric acid was given as a part of the cure. Wolpe (1914) reports achylia in all cases of exophthalmic goiter. Neilson (1914) found hyperacidity in cases of hyperthyroidism. It is also interesting to note that Katz (1920) reports that in many cases of hypothyroidism, hyperacidity and gastric and duodenal ulcers were found. In these cases thyroid feeding was found to be beneficial, reducing the acidity to normal and improving the general condition; this thyroid diet was used for ulcer treatment also. Hutton (1922) reports that gastric and duodenal ulcers are frequent in hypothyroid cases. Lorenzi (1922) studied the effect of thyroid feeding on the emptying time of the stomach and found the average time to be about two hours and fifteen minutes less than the average normal. Nothing was recorded in his paper as to the gastric secretion, but it is known that in cases of achylia or hypoacidity, the emptying time of the stomach is usually decreased.

METHOD. Pawlow pouch dogs were used in the experiment, complete results being obtained on three dogs. After allowing the proper length of time to pass after the operation for the gastric secretion to become normal, ten to fifteen controls were run on each dog. Each control consisted of testing the continuous secretion each hour for two hours, after which a test meal was fed. The test meal consisted of 250 grams of raw meat, 200 cc. of water and 200 cc. of milk. The secretion was then collected hourly for four hours after the meal. In each case the amount, free and total acidity (clinical units and pepsin content, Mett's method) were recorded. These tests were conducted four days a week, the other three days the dogs being kept on the stock diet. The dogs were in excellent condition at the end of the controls.

On completing the controls thyroid feeding was begun. The tests were conducted and the dogs fed exactly in the same manner as in the control tests with the exception of giving them the desired amounts of desiccated thyroid mixed with a small amount of water just before each meal. The thyroid was given daily for ten days beginning with 0.3 gram per kilo, or until a definite effect on the gastric secretion was produced in each dog. After a definite change in the secretion had been observed in each case, the thyroid feeding was stopped to allow the return of gastric secretion to normal. One gram of thyroid per kilo was fed daily in order to rapidly check the results previously found. The results obtained before were duplicated and the return to normal was observed.

Throughout the entire experiment the body weight, temperature, respiration, pulse and general condition of the animal were observed and recorded.

Results. The dogs appeared to be in a healthy condition throughout the five months' experiment, with a few exceptions. A few times in one dog vomiting occurred after a meal, but this did not seem to bear any special relation to the thyroid feeding at any time. The appetite was excellent throughout and seemed even greater during the period of the thyroid feeding. In each case it was noticed that mange became more severe and harder to check when thyroid was fed, the thyroid apparently causing the animal to be less resistant to this disease. No severe cases of diarrhea were noticed during thyroid feeding; however, there seemed to be a slight diarrheal condition in all three dogs the first three to five days after thyroid treatment, after which the dogs became normal. In two dogs restlessness was observed after feeding the larger doses of thyroid.

The body weight was decreased in all cases during thyroid feeding, though in one dog, which was given only 0.3 gram of thyroid, the loss was less than one kilo and later when the dog was not being given thyroid the same drop in weight was again noticed. In two of the dogs, however, the loss in weight was very definite both during the first period of thyroid feeding and the second. One of these dogs showed a gradual decrease in

weight amounting to 1.8 kilos during the first period of thyroid feeding with a return to 400 grams above normal in thirteen days after stopping the thyroid. During the second thyroid period a loss of 1.62 kilos in three

TABLE 1

Pawlow pouch dog

PROCEDURE	AMOUNT IN CUBIC CENTIMETERS			FREE ACID			TOTAL ACID		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
10 controls	5.5	14.8	8.7	18.1	86.1	41.2	37.0	95.6	56.1
Fed 0.3 gram thyroid from February 8–19	3.5	9.5	6.0	0	45.9	15.3	2.5	65.6	27.7
Stopped thyroid feeding on February 20									
February 24			7.8			39.4			55.6
March 2			8.0			29.4			51.9

Amounts of thyroid are per kilo body weight.

TABLE 2

Pawlow pouch dog

PROCEDURE	AMOUNT IN CUBIC CENTIMETERS			FREE ACID			TOTAL ACID		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
11 controls	17.8	30.2	23.3	67.5	117.5	98.2	88.7	124.4	108.8
Fed 0.3 gram thyroid from February 8–18–7 tests	10.7	27.9	18.0	50.1	99.4	80.7	62.5	106.2	93.8
Fed 0.6 gram thyroid from February 20–29, 4 tests	10.3	18.6	14.5	60.0	80.6	69.7	67.5	87.5	78.0
Fed 0.9 gram thyroid from February 29–March 14, 5 tests	10.7	19.0	11.1	60.6	75.6	66.8	70.0	86.5	77.1
Feeding continuous March 23–March 27, 4 tests	7.1	10.2	8.4	28.1	45.6	37.0	48.1	60.1	51.9
Stopped feeding March 27–April 1–16, 7 tests	12.5	31.6	24.5	63.1	104.4	87.3	76.2	112.5	97.1
Fed 1 gram thyroid from April 19–April 30–6 tests	8.8	18.0	13.3	14.4	58.7	37.6	37.5	74.4	41.7
Stopped feeding May 7–May 14			35.5			92.0			104.0

Amounts of thyroid are per kilo body weight.

weeks was observed with a gain of 600 grams in the first week following withdrawal of the thyroid extract. The third dog showed a loss in weight of 1.37 kilos during the first period and 1.22 kilos in the second with slight gain during the period when no thyroid was fed.

The pulse rate was increased in all cases during thyroid feeding. The normal rate of all three dogs, ranging between 59 to 65, was raised to an average of 84, 85 and 86 during the first ten days of feeding three-tenths of a gram per kilo. This average was raised to 99 and 106 in the two dogs receiving increased doses of thyroid and the return to a normal pulse in less than two weeks was noted in both cases.

The respiratory rate did not change beyond normal variations except in one case in which the respiratory rate was very fast near the end of one thyroid feeding period going as high as 86 per minute, the temperature

TABLE 3

Pawlow pouch dog

PROCEDURE	AMOUNT IN CUBIC CENTIMETERS			FREE ACID			TOTAL ACID			PEPSIN
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	
										mm.
12 controls	11.9	29.0	19.2	42.0	101.9	73.0	69.0	118.0	96.7	46.4
Fed 0.3 gram thyroid February 20–29—5 tests	10.8	18.0	13.1	46.9	63.7	53.9	61.9	77.0	71.8	26.0
Fed 0.6 gram thyroid February 29–March 24, 6 tests	6.2	11.0	9.0	15.6	76.2	29.1	28.7	86.2	52.6	33.0
March 24–27, 3 tests	8.0	8.7	8.3	0	0	0	11.9	21.9	17.5	27.5
Stopped feeding thyroid on March 8										
April 1–9, 3 tests	13.3	18.4	15.4	5.6	10.0	7.9	30.0	43.7	34.9	21.3
April 9–16, 4 tests	12.3	18.4	16.4	51.0	89.4	75.9	68.1	100.0	90.0	25.5
Fed 0.9 gram thyroid April 17–24	7.3	12.5	9.9	42.6	76.0	59.3	65.6	84.4	75.0	
April 29–May 6	4.3	8.7	6.2	0	21.9	9.9	21.1	47.5	35.4	24.0
Thyroid feeding stopped on May 7–May 14			15.8			69.4			82.0	

Amounts of thyroid extract are per kilo body weight.

at this time being 104. When thyroid feeding was stopped in this dog the respiratory rate went back to normal within two weeks' time. There was a slight rise in temperature in both cases where the larger doses of thyroid were given. The normal temperature ranged between 101° and 102.5°, whereas during thyroid feeding the temperature was usually between 103° and 104°. The proper amount of time which would be necessary for the best determination of pulse, respiration and temperature could not be given in this work, however, the results observed seem to indicate that daily thyroid feeding does influence the rate of pulse, respiration and degree of temperature.

There is no doubt as to the effect which daily thyroid feeding has on gastric secretion. The results show pronounced individual variations, but all definitely prove that desiccated thyroid when given in moderately large doses for the dog reduces the gastric secretion in amount and in free and total acidity.

Although all the data recorded cannot be given here, the tables below give a fairly good idea of the changes observed in the gastric secretion following a meal caused by feeding desiccated thyroid.

Throughout the entire experimental period the continuous secretion varied between wide limits both in amount of secretion and total acidity. However, averages made on the amount of secretion during the normal periods and the thyroid feeding periods showed a definite decrease in all cases, the decrease amounting to just one-half the normal in two of the dogs. No free acidity was present in any case in the continuous secretions. The total acidity was reduced in every case till there was practically no acid found in the very small amount of thick mucus which was secreted. In one dog it was interesting to note that the greatest decrease in the amount and total acidity came during the feeding of 0.6 gram of thyroid and then increased again to practically normal although the thyroid given was increased to 0.9 gram per kilo, which in the other two dogs the lowest acidity of the continuous secretion came at the same time that the lowest secretions were observed after the meal.

The three dogs experimented on showed quite extreme individual variations in the amount of resistance to the thyroid as judged from its effects on the gastric secretion following a meal. In one case almost complete achlorhydria was produced within the first week after thyroid feeding was begun (table 1). In another case complete achlorhydria and then achylia occurred in a month after feeding 0.3 and 0.6 gram (table 3), the absence of free acid in the first hour being noted several times in the first two weeks. The third dog (table 2), however, showed no marked change in the gastric secretion until the amount of thyroid given had been increased to 0.9 gram and the feeding continued for six weeks. The first effect noticed was an absence of free acid from the first hour secretion and a general decrease in the other three hours. Thus the effect of the thyroid caused a gradual and steady decrease in the amount of secretion and in the free and total acidity. In all cases the return to normal was noted in about two weeks after stopping thyroid feeding.

The amount of pepsin as measured by the Mett's tube method varied so greatly in the normal, when the total amount found in the four hours was taken into consideration, that the results were not at all satisfactory. In one case in which the readings were more constant during thyroid feeding (table 3), there was an increased amount of pepsin digestion per cubic centimeter, but decreased amount when the total secretion was considered.

DISCUSSION. Carlson (1924) states that thyroid produces a substance of specific physiological importance, stable in vitro, not destroyed by digestive juices or bacteria and absorbed in active form into the blood. Thus when thyroid is given by mouth as in these experiments it would seem probable that the symptoms of hyperthyroidism would be produced, if the symptoms of hyperthyroidism are due to a hyperactivity or hypersecretion of the thyroid glands.

Carlson also states that man is more susceptible to the toxic effect of thyroid administration than any other animal and that excessive thyroid feeding in man duplicates most of the symptoms of exophthalmic goiter. Thus it would seem that large doses of thyroid would be necessary to produce these symptoms in dogs. The loss of weight in the dogs studied was certainly not due to loss in appetite, but may be a secondary effect of the increased metabolism. The other symptoms as increased nervousness, increased pulse rate and temperature may also be only secondary effects of the disturbed metabolism, but are factors that affect gastric secretion in a negative manner.

Just how the thyroid extract affects the secreting cells of the gastric glands is not known. The results obtained, however, show that the hyposecretion and achylia produced by feeding thyroid daily is not permanent and thus the excess amount absorbed does not permanently injure the cells. Since the effect produced was a very gradual one, it might also be only a secondary result of disturbed metabolism and other abnormal conditions of the animal which might produce change in blood constituents thus affecting the activity of the secreting cells. It has been shown by Kuriyama (1918) that thyroid feeding affects the carbohydrate metabolism, decreasing the amount of glycogen stored by the liver and by Rhode and Stockholm (1919) that nitrogen elimination is increased. These and other unknown effects may cause a decided change in the blood. Another possible explanation would be that the thyroid secretion has a direct effect on the secreting cells under normal condition, in some way regulating the formation of the gastric secretion just as it regulates the amount of metabolism. Thus, in hypothyroidism where there is decreased amount of the active hormone circulating in the blood, the gastric secretion may be high, and where hyperthyroidism exists, the gastric secretion is low. This might show the existence of an indirect interrelationship in the activity of these two glands. The excessive amount of thyroid administered might cause the production of some toxic substance which ordinarily may be taken care of and excreted by the body, but which may now be formed in excess of that amount which can be disposed of and thus circulating in the blood produce a toxic effect on the secreting cells.

The lowered secretion might be due to a disturbance of the nervous mechanism of gastric secretion. It is well known that nerves do play a

rôle in gastric secretion. Also, it is well established that the first phase of gastric secretion, the appetite secretion, is dependent on a nervous reflex mechanism. From the results it seems that the appetite secretion is the first to be affected, the secretion not appearing until the second hour after feeding. The dogs certainly have no loss in appetite, if appetite can be measured by the eagerness for and haste in eating a meal. The excessive amount of thyroid circulating in the blood, however, might have in some way inhibited the nervous impulses by affecting the central nervous system directly or reflexly and thus decrease the gastric secretion just as pain and emotion, or as drugs, such as atropine, depress the gastric secretion. Inhibitory secretory nerves might be stimulated or secretory nerves be inhibited by the thyroid in excessive amounts.

The disturbance of the gastric-intestinal tract and intestinal digestion following thyroid extract administration is an important fact that might entirely explain these observations.

Fever depresses the gastric secretion and thus the higher temperature produced might be a factor in the hyposecretion.

In view of the effects on gastric secretion found in this experiment and the conflicting clinical observations reported above, it would be of interest to see if, by experimentally producing hypothyroidism, the gastric secretion would be increased and ulcers formed. It would also be of interest to repeat these experiments, except that a smaller amount of thyroid be given daily over an extended period of time. By this procedure the experiment might more nearly duplicate the disease of hyperthyroidism or toxic goiter which is generally believed to be essentially a disease of chronic character extending over months or years.

CONCLUSION

1. Thyroid extract administered to dogs in the amounts given above caused a decrease in body weight, increase in rate of pulse, slight increase in body temperature, increased restlessness and slight diarrhea.

2. A decrease in continuous secretion of gastric juice was observed in all cases.

3. The gastric secretion following a meal was decreased during thyroid feeding, the rate and extent of the effect depending on the amount of the thyroid fed and individual resistance.

For his kindness and helpful suggestions throughout the work on this problem, I wish to acknowledge my indebtedness to Dr. A. C. Ivy.

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CALCIUM AND PHOSPHORUS CONTENT OF STRONG AND WEAK CHICKS FROM HENS WITH AND WITHOUT CALCIUM CARBONATE IN THEIR DIET[1]

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From the Kentucky Agricultural Experiment Station, Lexington

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It has been estimated that under ordinary conditions from 5 to 10 per cent of all chicks hatched are weaklings that cannot be raised successfully to maturity and for this reason are destroyed by the intelligent poultryman. The cause of this condition has been attributed, 1, to a tendency of hens which have been laying heavily during the winter to lay eggs having weak germs the subsequent spring; 2, to improper care of the breeding stock, such as improper housing, feeding and sanitary conditions, which undoubtedly have a weakening effect on the chicks; and 3, to improper conditions during incubation.

In an effort to throw more light on this complicated subject an experiment was planned to study from a chemical standpoint, the conditions resulting from the feeding of a grain-buttermilk ration, with and without a calcium carbonate supplement, to laying hens.

The authors have shown (1924) that when a calcium carbonate supplement such as oystershell or limestone is added to a grain-buttermilk ration for laying hens, the total number of eggs is greatly increased and the contents or edible portion of the eggs and the shells weigh distinctly more than those from similar hens having been fed the same ration without the calcium carbonate supplement, and that under the same conditions (1925a) both the yolks and whites are increased in size, when the calcium carbonate supplement is added. They have shown further (1925b) that the hatchability of eggs is markedly diminished by withholding the calcium carbonate supplement from the laying hens.

Cross (1912) has stated that "the weak chick is equally as rich in phosphorus as the strong chick. The chicks are designated weak and strong according to their ancestry. There is, of course, a possibility of the offspring of weak parents being strong and vice versa, so conclusive proof is not here shown," and finally concludes in the summary that "the weak chick is equally as rich in phosphorus as the strong chick."

[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station, Lexington.

On January 1, 1925, the date of the beginning of the experiment described herein, 30 White Leghorn hens were weighed individually and divided into three groups of 10 each designated lots 1, 2 and 3. The lots were divided with the view of equalizing their weights and egg-laying capacities. The egg records for the pullet year showed that the average for each lot was 155, 159 and 157 eggs per hen, respectively. The average weight of the hens in each lot was 1667, 1698 and 1715 grams, respectively, on the day the experiment started. The hens were the same age, having been hatched at the same time and they had a common ancestry. From the time of hatching until the start of the experiment all the hens had been kept under identical conditions in the same house.

On January 1, 1925, these three lots were placed in separate shed-roof houses that were identical in every respect, lot 1 being allowed a fair sized grass range and the other two lots being confined to their houses during the experiment. These houses were so constructed and placed as to permit the entrance of direct sunlight for several hours each day. A trap-nest record was kept of the eggs laid during the experiment and the eggs were weighed individually.

All three lots were given a ration consisting of wheat, yellow corn and buttermilk, the last *ad libitum*. In the mornings and evenings the grain was thrown into the oat-straw litter which was changed frequently. Grit that contained no acid-soluble calcium compounds was fed at the rate of 20 grams per hen per month and no water was given the hens, thereby forcing the consumption of buttermilk. Calcium carbonate in the form of oyster-shell was fed *ad libitum* to lots 1 and 2 but withheld from lot 3, during the experiment.

On February 1, 1925, three strong White Leghorn cocks whose fertilizing powers were known to be high, were placed in the three pens and were changed every two weeks from pen to pen, thereby equalizing the influence of the male birds.

Beginning March 1, 1925, the eggs were saved for hatching. At this time the average weight of the dry eggshell from each lot was 6.2, 5.7 and 3.9 grams, respectively. All the eggs laid during the two weeks previous to March 14, 1925, by all three lots, were weighed individually, placed in an electric incubator and allowed to incubate normally. This was repeated for two subsequent hatchings which were set on April 11 and May 4. On the 18th day of the incubations the infertile and dead-germ eggs were removed and the eggs remaining were sacked individually and allowed to hatch. As soon as the chicks were dry, they were removed from the incubator that had been kept dark to keep the chicks from eating the broken shell. The shells from each lot were weighed collectively, after drying to a constant weight at 100°C. in an electric oven, and the average weight of one dry shell calculated. The average dry weight of the shell

was subtracted from the original average weight of an egg in each, which gave the average weight of the contents of the eggs of each lot.

The chicks were then examined and divided into lots, classed as weak and strong chicks. The weak chicks were those that seemed to lack vigor and vitality and moved about with difficulty, remaining on the floor except when disturbed; the others were classed as strong. The weak and strong chicks in each lot were placed in separate dishes, chloroformed and dried to a constant weight at 100°C. and the average weight of the dried carcass was obtained from this.

TABLE 1

Average weights of the eggs, shells, contents and the dried chicks, expressed in grams

	FIRST HATCH MARCH 14, 1925		SECOND HATCH APRIL 11, 1925		THIRD HATCH MAY 21, 1925
	Strong chicks	Weak chicks	Strong chicks	Weak chicks	Strong chicks
Lot 1, $CaCO_3$ and range:					
Number of eggs hatched	8.0	—	7.0	3.0	9.0
Average original weight of 1 egg	60.2	—	59.4	59.3	59.0
Average weight of dried shell after hatching,	6.0	—	5.9	6.0	5.8
Average weight of contents	54.2	—	53.5	53.3	53.2
Average weight of 1 carcass, dried at 100°	11.0	—	9.2	10.7	11.0
Lot 2, $CaCO_3$, no range:					
Number of eggs hatched	8.0	4.0	8.0	—	9.0
Average original weight of 1 egg	59.3	59.2	60.0	—	58.9
Average weight of dried shell after hatching	6.1	5.7	6.0	—	5.9
Average weight of contents	53.2	53.5	54.0	—	53.0
Average weight of 1 carcass, dried at 100°	10.5	10.8	9.5	—	9.5
Lot 3, neither $CaCO_3$ nor range:					
Number of eggs hatched	8.0	—	7.0	—	5.0
Average original weight of 1 egg	56.0	—	55.4	—	55.0
Average weight of dried shell after hatching	4.2	—	3 9	—	4.0
Average weight of contents	51.8	—	51.5	—	51.0
Average weight of 1 carcass, dried at 100°	10.8	—	8.8	—	8.0

It will be seen in table 1 that there were 3 weak chicks in the second hatch of lot 1 and 4 in the first hatch of lot 2 and that there were no weak chicks in any of the hatches in lot 3. Here we see that the average weights of the eggs in lots 1 and 2 were approximately 4 grams heavier than those in lot 3, and that the average weights of the dry shells in lots 1 and 2 were approximately 2 grams heavier than in lot 3. The average weights of the contents were approximately 2 grams heavier in lots 1 and 2 than in lot 3. Finally, we see that the average dry weights of the chicks were 10.5 and 10.1 grams for lots 1 and 2, respectively, and 9.2 grams for lot 3. It is shown clearly that the weights of the eggs, shells, contents and dry weight of the chicks are notably heavier when produced by hens receiving a cal-

cium carbonate supplement in a ration such as given here than when calcium carbonate is absent. It is especially noticeable that while there were no weakling chicks in lot 3 yet those that hatched in this lot were not only lighter in weight but notably less vigorous than the strong chicks of the other two lots. It is to be noted, also, that the average weight of a dried chick is less in the second and third hatches of lot 3 than in the first hatch.

After the dry weight of the composite sample of the chicks of each lot was obtained, the samples were ashed separately in quartz dishes and the resulting ash dissolved in dilute HCl, made to 100 cc. with distilled water and calcium and phosphorus were determined in separate aliquots, the former according to McCrudden (1909) and the latter by the volumetric method of the Association of Official Agricultural Chemists (1919). These percentages were calculated to the dry weights of the chicks.

In table 2 will be seen the percentages and weights of CaO and P_2O_5 in the dried carcasses of the chicks from each lot. The percentage and actual quantity of CaO are less in the weakling chicks of lots 1 and 2 than in the strong, while the percentage and weight of P_2O_5 in the weak chicks of these two lots are not noticeably lowered. This suggests that calcium in some form, possibly as phosphate, is deficient in the weakling chicks.

If the calcium be calculated as tricalcium phosphate, it will be seen that there is an excess of phosphorus over that required to form tricalcium phosphate and that there is a smaller excess in the chicks of lot 1 than in those of lot 2 and less in lot 2 than in lot 3.

Ratio of calcium to phosphorus. The figures in table 3 were obtained by dividing the weights of calcium, computed from the weights of CaO given in table 2, by the corresponding weights of phosphorus, computed from the weights of P_2O_5 given in the same table. The strong chicks of lot 1 contained relatively more calcium, as compared with the phosphorus content, than those of the other two lots. The proportion in the weak chicks of this lot, however, is commensurate with that in the chicks of the other lots. Again, as the laying season progressed, the proportion of calcium increased, in the strong chicks of lot 1, a relation that is not apparent in the chicks of the other lots. Inasmuch as the only difference between lots 1 and 2 is that the mother hens of lot 1 had range, whereas those of lot 2 were confined to the house, the inference is that something connected with the range enhanced the ability of the developing chicks to appropriate calcium, while still within the shell. Important factors which may have combined to bring about this result are the free exposure to daylight of the hens of lot 1, their greater opportunity for exercise and the possible addition of insects and vegetation to their diet. The possibility of their getting mineral matter of a different kind from that given to the hens in confinement should be mentioned also.

TABLE 2

Calcium and phosphorus content of the chicks, expressed in grams

	FIRST HATCH MARCH 14, 1925		SECOND HATCH APRIL 11, 1925		THIRD HATCH MAY 4, 1925
	Strong chicks	Weak chicks	Strong chicks	Weak chicks	Strong chicks
Lot 1, $CaCO_3$ and range:					
Average percentage CaO in dried carcasses	2.24	—	2.27	1.77	2.50
Grams of CaO in 1 chick	0.2464	—	0.2088	0.1894	0.2750
Average percentage P_2O_5 in dried carcasses	2.50	—	2.40	2.38	2.35
Grams of P_2O_5 in 1 chick	0.2750	—	0.2208	0.2547	0.2585
Grams of P in excess of that required to form $Ca_3P_2O_8$ with the CaO	0.0294	—	0.0238	0.0414	0.0115
Equivalent percentage in dried carcasses	0.27	—	0.26	0.39	0.11
Percentage of the total P	24.0	—	25.0	37.0	10.0
Lot 2, $CaCO_3$, no range:					
Average percentage CaO in dried carcasses	1.88	1.61	2.12	—	1.87
Grams of CaO in 1 chick	0.1974	0.1735	0.2114	—	0.1776
Average percentage P_2O_5 in dried carcasses	2.52	2.36	2.73	—	2.60
Grams of P_2O_5 in 1 chick	0.2646	0.2549	0.2594	—	0.2470
Grams of P in excess of that required to form $Ca_3P_2O_8$ with the CaO	0.0427	0.0473	0.0353	—	0.0423
Equivalent percentage in dried carcasses	0.41	0.44	0.37	—	0.45
Percentage of the total P	37.0	42.0	31.0	—	39.0
Lot 3, neither $CaCO_3$ nor range:					
Average percentage CaO in dried carcasses	1.73	—	1.90	—	2.00
Grams of CaO in 1 chick	0.1868	—	0.1672	—	0.1600
Average percentage P_2O_5 in dried carcasses	2.21	—	2.55	—	2.68
Grams P_2O_5 in 1 chick	0.2387	—	0.2244	—	0.2144
Grams P in excess of that required to form $Ca_3P_2O_8$ with the CaO	0.0353	—	0.0363	—	0.0346
Equivalent percentage in dried carcasses	0.32	—	0.41	—	0.43
Percentage of the total P	34.0	—	37.0	—	37.0

TABLE 3

Weight of Ca in the chicks, taking the weight of P as unity

	STRONG CHICKS			WEAK CHICKS	
	First hatch	Second hatch	Third hatch	First hatch	Second hatch
Lot 1—Mothers had $CaCO_3$ and range	1.465	1.548	1.739	—	1.215
Lot 2—Mothers had $CaCO_3$ without range	1.222	1.331	1.176	1.113	—
Lot 3—Mothers had neither $CaCO_3$ nor range	1.280	1.218	1.220	—	—

The ratio of phosphorus to calcium is sensibly the same in lot 2 as in lot 3, and is fairly uniform for the three hatchings.

The ratio of phosphorus to calcium in tricalcium phosphate is 1:1.936.

Weights of CaO assimilated from the shell, by the chicks. The average percentage of CaO in the contents of the eggs of White Leghorns has been found to be close to 0.07 per cent (1923). Using this figure in combination with the weight of contents given in table 2, to compute the weights of CaO originally present in the contents, and subtracting these from the average weights of CaO found in the chicks, taken from table 1, gives an approximation of the average weights of CaO derived from the eggshell, by the chicks of the several lots. As seen in table 4, the chicks of lot 1 took more CaO from the shell, as a rule, than those of lot 2, and the chicks of lot 2 took more than those of lot 3. This shows in another way that the chicks of lot 1 were more efficient in acquiring calcium than those of the other two lots and, further, that those of lot 3 were the least efficient of all.

TABLE 4

Weight of CaO acquired from the shell. Grams

	STRONG CHICKS			WEAK CHICKS	
	First hatch	Second hatch	Third hatch	First hatch	Second hatch
Lot 1—Mothers had $CaCO_3$ and range	0.2085	0.1703	0.2378	—	0.1894
Lot 2—Mothers had $CaCO_3$ without range	0.1602	0.1736	0.1405	0.1361	—
Lot 3—Mothers had neither $CaCO_3$ nor range	0.1505	0.1311	0.1243	—	—

A falling off of the CaO taken up, as the laying period progressed, is also apparent in lots 2 and 3. Inasmuch as the weight of CaO used by the chick is small as compared with the weight of even the lightest shell, the question arises as to why these chicks did not develop to approximately the same size in all lots and take sensibly the same weights of CaO from the shells. Simple shortage of calcium in the environment of the developing chick seems not sufficient to account for the differences, because an excess of eggshell was present in each case. Apparently the conditions affecting the hens when the eggs were produced have left a subsequent effect upon the developing embryo.

Of course the weights of $CaCO_3$ corresponding to these weights of CaO should be added to the observed weights of shell, thus diminishing correspondingly the weights of contents stated in table 1. The change in weight of contents will be inconsiderable, however, the amounts to be deducted ranging from about 0.2 gram to about 0.4 gram.

SUMMARY

In briefly summing up these results it seems that the addition of a calcium carbonate supplement such as ground oystershell, increases the weight of the eggs, shell, contents and dry weight of the chicks hatched.

The quantity and percentage of phosphorus are approximately the same in strong chicks as in weak chicks.

The quantity and percentage of calcium are less in weak chicks than in strong chicks.

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STUDIES ON THE EFFECTS OF BATHS ON MAN

III. Effects of Hot Baths on Respiration, Blood and Urine

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The literature dealing with this general subject is given in detail in the first paper of the series (Bazett, 1924) and is not repeated here. In every case the effects of hot baths have included a marked hyperpnea and, when measured, a considerable drop in alveolar carbon dioxide percentage. J. B. Haldane (1905) and Sutton (1909) found similar effects in hot rooms showing that the respiratory reaction to hot baths is largely due to the change in body temperature. A change in the pH of the blood is inferred by Haggard (1920), who finds there is not sufficient decrease in the alkali of the blood to compensate for the considerable fall in alveolar carbon dioxide, and was demonstrated directly by Cajori, Crouter and Pemberton (1923) who describe a measurable increase in the alkalinity of the blood. Koehler (1923) also observed an increase in blood pH after submersion in warm water. A diuresis was found by Bazett, Thurlow, Crowell and Stewart (1924) to be present in baths of neutral temperature as well as in hot baths. According to Griffiths and Hansell (1925) this is probably induced by the hydrostatic pressure exerted on the abdomen.

This paper is a report of six experiments, in which hot baths were continued until a state of tetany developed, on three human subjects and in which observations were made on the change in body temperature; the rate, depth and minute volume of respiration; the quantity, titratable acidity, ammonia and pH of urine, as well as on the pH and total carbon dioxide content of the blood. From analyses of expired air computations have been made of oxygen consumption, respiratory quotient, and of the approximate alveolar carbon dioxide tension, which has been checked by computations also from blood figures.

Methods and technic. The subjects had breakfast at about 8 a.m. and no food or drink from then till the end of the experiment, which usually was started at 2 p.m. They were immersed up to the neck in a bath five feet long provided with a stirrer to keep the water in constant circulation. The addition of hot water from a tank by the release of a magnetically operated valve under the control of a thermostatic Hearson capsule maintained a constant temperature.

TABLE 1

Protocol of experiment 3

Subject H.C.B. Sex—male. Weight 65.0 K.

Average rate of rise of temperature—4.1 degrees per hour

			RESPIRATION						URINE				BLOOD	
TIME	BATH TEMPERATURE	BODY TEMPERATURE	Respirations per minute	Tidal air	Ventilation	Respiratory quotient	O_2 consumed	Alveolar CO_2 tension	Urine per hour	0.1 N Acid per 100 cc.	0.1 N NH_3 per 100 cc.	pH	Plasma pH	Whole blood total CO_2
				cc.	*liters per min.*		*cc./ min.*	*mm.*	*cc.*					
2:00									84	18.7	15.2	5.5	7.49	54.9
2:05														
2:10	36.6	37.0												
2:15	36.6	37.1												
2:20	36.6	37.3												
2:25	36.7	37.3												
2:30	36.4	37.4												
2:35	36.3	37.4												
2:40	36.1	37.4												
2:45	35.9	37.4	25	583	7.13	0.83	295	41.6						
2:50	35.7	37.3											7.505	54.4
2:55	35.9	37.3												
3.00	35.7	37.3												
3:05	36.1	37.3												
3:10	36.4	37.3												
3:15	36.3	37.2	28	618	8.33	0.82	361	38.3	180	15.9	13.0	7.4		
3:18	37.4	27.3												
3:20	40.5	37.2												
3:21	41.3													
3:22	41.7													
3:25	41.6	37.6												
3:30	41.9	38.1	24	1993	20.80	1.22	482	23.0					7.64	41.5
3:35	41.7	38.7												
3:37	42.2													
3:40	42.5	39.3	29	1556	21.64	1.10	541	18.7						
3:45	43.0	39.5											7.59	37.9
3:50	42.9	39.6							82	2.9	5.9	7.4		
3:55	36.5	39.7												
4:00	34.8	39.5	33											
4:05	34.8	39.5												
4:10	36.6	38.7												
4:15	36.6	38.5	29	487	8.28	0.79	360	42.7						
4:20	36.4	38.3												
4:25	36.3	38.2							29	0.7	2.2	7.85		
4:30	36.3	38.1											7.49	53.8
4:35														
4:40														
4:45														

The bath temperature was recorded at least every five minutes, and more frequently during the periods of rapid change. After the subject had been immersed for a control period of one hour in a neutral bath (36.0 to 36.5°C.) the temperature was rapidly elevated to maxima of from 40.2 to 43.0 degrees, and maintained at that level for thirty to sixty-five minutes, depending on the symptoms.

Body temperature was determined by a rectal thermometer of the automatically recording resistance type described by Woodhead and Varrier Jones (1916) with occasional checks by comparison with the temperature of urine as passed. Expired air was collected in a Tissot spirometer as modified by Drinker. A recording mechanism provided graphic records of ventilation during the period of collection. A weighted cord was attached to the bell of the spirometer and passed over a large wooden pulley mounted on ball bearings; a second smaller pulley attached to the first and rotating with it raised a writing point vertically over a slowly moving smoked drum, so recording the rate at which the spirometer bell rose. The respiratory rate and tidal air were computed from these records.

Douglas valves with a dead space of 60 cc. were used in the collection of expired air, which was analyzed by the Haldane-Henderson apparatus. The pH of the blood was determined by Cullen's colorimetric method. There was, however, some slight loss of CO_2 during centrifugalisation as, though this was done in corked centrifuge tubes under oil, the tubes were not filled to the brim. Van Slyke's apparatus, with constant pressure readings, was used to measure the total carbon dioxide content of the blood.

The reasons for reporting this comparatively small number of experiments are chiefly the severity of the after-symptoms and the possible danger of the experiment itself. In five of the six periods (all except expt. 5) clearly defined tetany was obtained. The immediate and more startling effects subsided on lowering bath temperature but the subject always complained of after symptoms for some hours, and in two cases, even longer.

The results of each experiment were summarized in protocols of the type shown below for experiment 3 (table 1). In addition for comparative purposes they were also charted graphically as shown for experiments 2 and 6 (figs. 4 and 5).

Remarks. Control urine taken from 1:00 to 2:00 p.m.

1:00 to 2:08 p.m.	Lying down in the room
2:08 p.m.	Entered neutral bath
3:18	Temperature of bath raised
3:22	Sweating
3:25	Deep, slow, heavy breathing observed
3:29	Tingling,—first signs of tetany
3:37	Breathing very deep and fairly slow

3:40	Marked tingling
3:50	Severe tetany with diaphragmatic spasm and marked difficulty in breathing
3:52	Bath temperature dropped
3:54	Breathed carbon dioxide and felt better temporarily. Oxygen had no effect
4:05	Shivering
4:30	Out of bath

In this particular experiment the subject had fasted since the previous evening except for 100 cc. of water and one slice of bread and butter for breakfast at 8:00 a.m.

RESULTS. Body temperature increased at rates varying from 1.9 to 5.3 degrees per hour. The rate of rise was greatest at the beginning of each experiment when body and bath temperatures differed most. Ordinarily the fall in temperature was rapid as soon as cold water was added.

Respiration. In all the experiments total ventilation increased steadily as body temperature rose. It is primarily affected by the rate of rise of body temperature. Figure 1 shows a comparison of two records on the same subject (J.W.D.) demonstrating that with a rectal temperature of about 39.5 degrees the ventilation rate was 15.6 liters per minute in experiment 1, where the rate of temperature rise was 2.4 degrees per hour, while ventilation was about 18.7 liters per minute at the same temperature level where the rate of rise was 5.3 degrees per hour. This relation between rate of rise of body temperature and total ventilation appears more definite in table 2, in which the experiments have been arranged in series according to the steepness of the temperature rise.

The increase in minute volume is relatively slow at first, but as body temperature reaches higher levels, even though the rate of rise becomes slower, the gradient of increase in ventilation is often steeper. Figure 2 (again of subject J.W.D.) shows an experiment extending over 65 minutes during which time the rate of rise of body temperature is slow, 1.9 degrees per hour, and relatively uniform. As body temperature rises total ventilation becomes greater to an increasing degree, though the rate of rise of temperature remains fairly constant throughout.

Therefore it appears that the increased ventilation during a period of rise of body temperature in the hot bath is due partly to the rate of rise of temperature and partly to the absolute level of body temperature.

Total ventilation in general increases steadily throughout the period of rise of temperature. Tidal air and rate of respiration vary in magnitude. The first effect is, in the majority of cases, an increased tidal air,—a greater depth of breathing,—accompanied by an increase in total ventilation while the respiratory rate remains almost constant or may even fall. At first therefore increased ventilation usually results from increased amplitude.

After this first rise in tidal air there is usually a marked fall, at which time

the still further increased total ventilation is apparently obtained by a rise in respiratory rate. During the latter part of the experimental period both rate and depth increase together. Likewise if the rate of rise of body temperature is very rapid they rise together from the beginning.

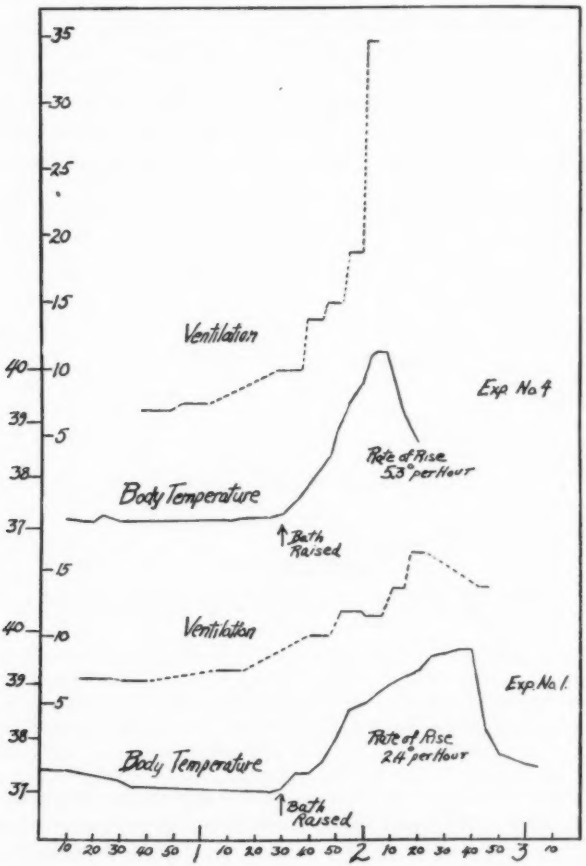

Fig. 1. Chart showing relation of total ventilation of one subject (J.D.) to the rate of body temperature increase. In experiment 4, body temperature rose at the rate of 5.3°C. per hour; in experiment 1, 2.4 degrees per hour. Ordinates show degrees Centigrade and liters per minute, abscissae time in minutes.

Table 2 shows a summary of the results, giving the highest minute volume, tidal air, respiratory rate, and the lowest computed alveolar carbon dioxide for each experimental period. Except for ventilation there is

apparently little correlation of these factors separately with the rate of rise of body temperature.

During the period of recovery conditions are reversed. The first reaction to fall in body temperature is mainly a decreased depth of respiration, respiratory rate remaining high, or even increasing slightly (three experiments), while the tidal air falls rapidly to normal or below. The resulting lower alveolar ventilation helps to restore a normal alveolar carbon dioxide tension, in spite of the fast respiratory rate.

The graphic records made by the recording spirometer during the period of hyperpnea sometimes show a tendency to rhythmic variations in ventilation of the Cheyne-Stokes type (fig. 3). No definite Cheyne-Stokes

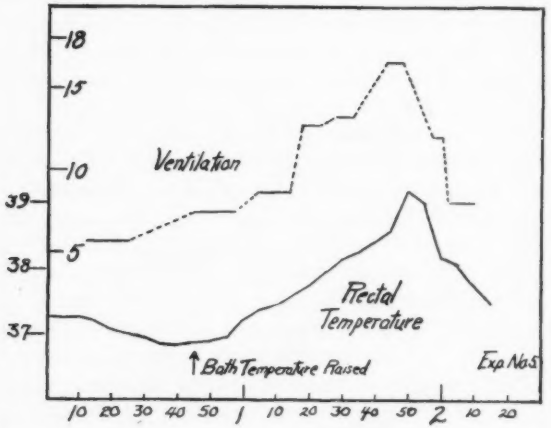

Fig. 2. Chart showing the tendency toward a more rapid increase in total ventilation as absolute body temperature becomes greater, though the rate of increase of body temperature is practically constant throughout. Experiment 5, Subject J. D. Ordinates—liters per minute, and degrees Centigrade. Abscissae—time in minutes.

respiration was observed, however. In the record the lower line shows normal ventilation, the upper shows the extreme hyperpnea existing at the climax of the experiment. Three regions of slightly increased tidal air are shown.

The respiratory quotient in all cases followed the ventilation curve, with values during the period of greatest hyperpnea of from 1.05 to 1.40, having risen from normals between 0.75 and 0.85. After the fall in temperature there is a decrease in respiratory quotient, the lowest observed being 0.62.

Oxygen consumption was found to increase slightly in the bath even at neutral temperatures since in all cases where two control samples were

taken before raising the bath temperature the second gave the higher value. A second rise, much more marked, was observed when the temperature was raised and when respiratory movements became more active, especially in the acutely hyperpneic periods.

TABLE 2

EXPERIMENT NUMBER	SUBJECT	RATE OF RISE OF BODY TEMPERATURE	HIGHEST BODY TEMPERATURE REACHED	HIGHEST VENTILATION LIT./MIN.	HIGHEST TIDAL AIR	HIGHEST RESPIRATORY RATE	LOWEST ALVEOLAR CO_2
					cc.		
4	J. D.	5.3	40.3	34.4	1376	29	20.1
6	H. C. B.	4.5	40.4	24.9	1764	20	15.6
3	H. C. B.	4.1	39.7	21.6	1993	24	18.7
2	U. M.	4.1	39.5	14.4	897	16	19.3
1	J.D.	2.4	39.7	16.1	982	28	15.6
5	J.D.	1.9	39.2	16.6	995	21	22.6

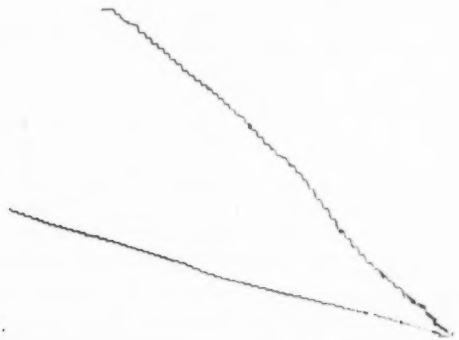

Fig. 3. Tracing from recording spirometer showing tendency during hyperpnea toward Cheyne-Stokes respiration. The lower line represents normal respiration during the control period in the neutral bath. The upper record shows the marked hypernea resulting after thirty minutes in the hot bath. Three zones of increased tidal air are shown, separated by two regions of slightly diminished depth of respiration. Record reading from right to left.

The approximate alveolar carbon dioxide tension has been calculated from the percentage of carbon dioxide in the expired air, the dead space, and tidal air by the following formula:

$$\frac{\text{Tidal air}}{\text{Tidal air} - 200\text{ cc.}} \times \text{\% carbon dioxide in expired air} \times \text{Barometric pressure corrected for Water vapor at 37 degrees} = \text{Carbon dioxide tension}$$

It is assumed that the dead space remains relatively constant at 140 cc., the Douglas valves having a volume of 60 cc. thus making a total dead space of 200 cc. Calculations show that with the large tidal air figures obtained in our experiments, even though the dead space increases as claimed by Douglas and Haldane (1912), little difference in the final values would result. No allowance has been made for the slight changes in vapor tension with change in body temperature.

In some of our experiments both blood pH and total carbon dioxide content were determined. In experiments 4, 5 and 6 plasma CO_2 was measured, and in experiment 3 that of whole blood. The venous carbon dioxide tensions of the arm blood, calculated from these quantities are of the same order of magnitude as those calculated from the respiratory data (table 3). The difference is relatively small and usually in the same direction, the blood carbon dioxide tension being slightly less than the computed alveolar carbon dioxide tension both before and after submer-

TABLE 3

EXPERIMENT NUMBER	SUBJECT	RATE OF RISE OF BODY TEMPERATURE	VENOUS CO_2 TENSION	ALVEOLAR CO_2 TENSION	GREATEST TOTAL VENTILATION	HIGHEST TIDAL AIR	HIGHEST RESPIRATORY RATE	DURATION OF EXPERIMENT
								minutes
4	J.D.	5.3	16.4	20.1	34.4	1378	29	35
6	H.C.B.	4.5	22.1	21.3*	24.9	1764	20	35
3	H.C.B.	4.1		18.7	21.6	1993	24	30
2	U.M.	4.1		19.3	14.4	897	16	30
1	J.D.	2.4		15.6	16.1	982	28	60
5	J.D.	1.9	19.2	22.6	16.6	995	21	65

* Average of two figures.

sion. The carbon dioxide tensions calculated from respiratory data would be somewhat lowered if allowance were made for the change in vapor tension at the higher temperatures, but such an error is likely to be partly balanced by an opposite error resulting from a change in dead space. The blood in centrifugalisation probably lost a little carbon dioxide and this no doubt also contributes to the difference.

The value of pK (taken as 6.10) was at the time of the experiments unknown for the body temperatures observed in the bath. The recent figures of Austin and Cullen (1925) now permit the computation of the carbon dioxide tension of venous blood in the arm, making allowance for changes in pK with rise in body temperature. The recalculation of the blood data, correcting for changing pK and for the changing solubility of carbon dioxide with rising temperature, brought about a reduction in the difference between the two sets of figures. The correction was not great, but in each case increased the figures for the carbon dioxide tension of

venous blood by from 0.5 to 1.5 mm. Nevertheless in nearly all the computations the venous carbon dioxide tension even after correction is still less than the alveolar tension computed from respiratory data.

The only possible physiological reason for the lower blood figures would appear to be the loss of carbon dioxide from the blood flowing through the skin, and the errors already enumerated would prevent any deduction being made as to whether this did or did not occur. In any case good confirmation is given of the general degree to which the carbon dioxide tension may be lowered under these conditions. Koehler (1923) also records exceedingly low values for alveolar carbon dioxide tension during submersion in hot baths.

References to the figures (figs. 4 and 5) show that there is a steady fall in alveolar carbon dioxide tension depending on the degree of over-ventilation. Table 3 gives a summary of the lowest values obtained in each experiment as computed from respiratory data. In the adjacent column are given the venous carbon dioxide tensions for blood at the corresponding period, as determined from pH and total carbon dioxide, corrected for the higher temperature, using the constants of Austin and Cullen (1925). In all comparisons were possible on thirteen blood samples and results were with one exception (expt. 6, shown in table 3) of the same general type, the venous figures being slightly but uniformly lower than those from respiratory data.

The factors which seem to influence the fall in alveolar carbon dioxide are chiefly the rate of ventilation, the type of breathing, and the length of the experimental period. When the temperature of the bath is lowered the alveolar carbon dioxide returns rapidly to a normal value, even while the respiratory rate is high, this being probably due to the shallow character of the respiration.

Blood. The pH change of the blood is apparently determined by the minute volume of respiration but is not proportional to it. It is probably dependent upon several factors among which may be the kidneys, the degree of sweating, and lactic acid formation. The last particularly may be important judging by the work of von Anrep and Cannan (1923).

The change in blood pH was always marked, varying from 0.12 to 0.33. As drawn from the arm vein the blood had a bright red color. That ventilation is not the only factor in producing these changes is shown by table 4 in which the greatest pH change occurred with the least hyperpnea, and the smallest pH change with the greatest ventilation. There is a much closer relationship between blood pH and alveolar carbon dioxide.

In the period of tetany there occurred on the two occasions when two blood samples were taken during the stage of severe symptoms (expts. 3 and 6) a fall of pH with a simultaneous fall in carbon dioxide content as shown in table 5. This could then only be due to an organic acid such

as lactic acid formed by the tissues and poured into the blood stream. Variations in such a reaction would also aid the explanation of the complex relationship of the effect of rate of rise and total duration on the severity of symptoms.

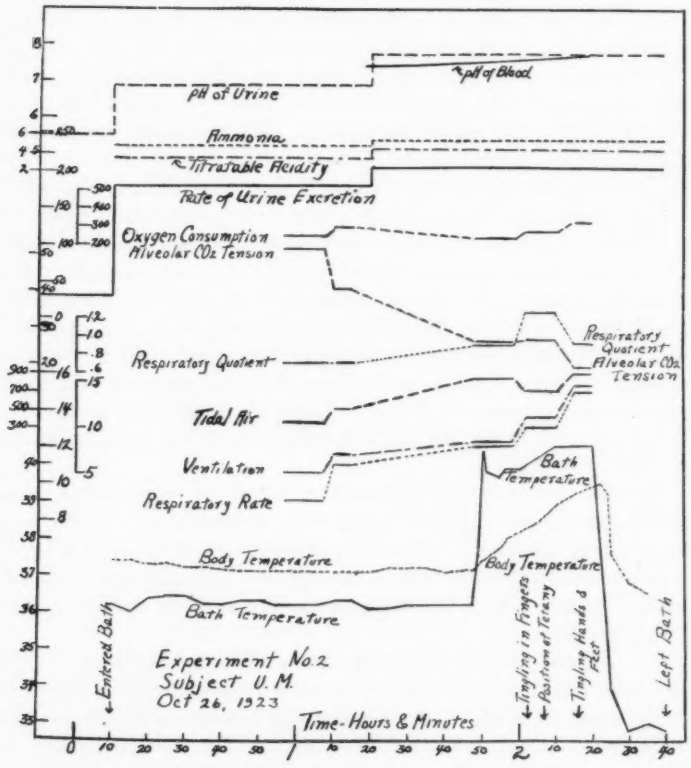

Fig. 4. Chart showing the complete results of experiment 2 (Subj. U. M.) in which the largest pH change (0.33) was observed. The immediate and delayed reactions were most marked. Ordinates show the scale corresponding to each curve. Abscissae give time in minutes.

The total carbon dioxide content of the blood was reduced in general according to the degree of hyperpnea.

Tetany. There is no evidence in these experiments of a critical level of pH at which tetany occurs. Tetany was obtained in all baths but the most marked and persistent example occurred with the blood pH change of 0.33. The change in oxygen tension which should result from such a

change in pH would probably be insufficient to be the direct cause of the symptoms. Thus relief of tetany was observed on the use of expired air, and also with mixtures of oxygen and carbon dioxide, but not with oxygen

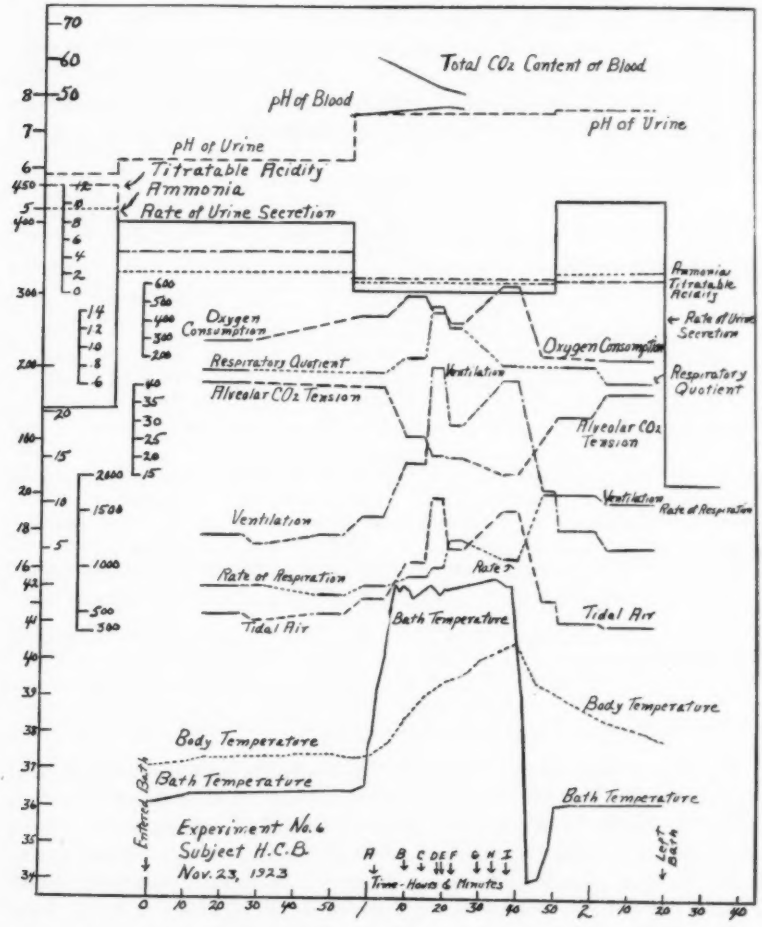

Fig. 5. Chart showing complete results of experiment 6 (Subj. H.C.B.) in which the pH change was only moderate in degree. Ordinates show the scale corresponding to each curve. Abscissae give time in minutes. *A*, sweating first observed; *B*, profuse sweating; *C*, tingling in finger-tips; *D*, noticeable hyperpnea; *E*, spasm of diaphragm; *F*, rectus and leg muscles in tetany; *G*, very strong general tetany; *H*, breathed oxygen but felt no improvement; *I*, breathed expired air and the arms became slightly relaxed and the subject felt more at ease.

alone. In one case a return into tetany was observed as the breathing of carbon dioxide was stopped.

The after-symptoms seem to be proportional to the pH change. The individuals more resistant to the change of blood pH recovered more quickly from the effects of tetany also.

Urine. The volume of urine secreted was usually definitely increased in addition to the diuresis characteristic of the first hour in the bath. This is probably due to the alkaline condition of the blood since a diuresis was also observed by Collip and Backus (1920) in purely voluntary hyperpnea outside a bath.

TABLE 4

EXPERIMENT NUMBER	SUBJECT	RISE IN BLOOD pH	RATE OF TEMPERATURE RISE	VENTILATION	ALVEOLAR CO_2	DURATION
2	U.M.	0.33	4.1	14.4	19.3	30
1	J.D.	0.23	2.4	16.1	15.6	60
6	H.C.B.	0.17	4.5	24.9	15.6	35
3	H.C.B.	0.14	4.1	21.6	18.6	30
5	J.D.	0.14	1.9	16.6	22.6	65
4	J.D.	0.12	5.3	34.4	20.1	35

TABLE 5

	TIME	BLOOD pH	TOTAL CO_2	SYMPTOMS
Experiment 3	3.29	7.64	41.5	Faint tingling
	3.50	7.59	37.9 (whole blood)	Marked and definite tetany
Experiment 6	3.19	7.64	52.3	Tingling began 3 minutes before
	3.25	7.62	50.5 (plasma)	After severe tetany

Titrable acidity fell uniformly in the hot bath with the exception of experiment 2. The subject used on this day had such a violent reaction (with the largest observed change in blood pH) accompanied by such acute symptoms and prolonged after-effects that he was not tested again. It is of interest to note that his was the only experiment in which the urine volume did not rise markedly with the onset of alkalosis and the urine actually increased slightly in titratable acidity, while ammonia excretion was practically unchanged (fig. 4). Presumably, therefore, the renal adaptation mechanism was not functioning properly in this instance.

Ammonia for the most part follows acidity very closely. There is a uniformly slightly smaller quantity of ammonia per given volume than acid. In five experiments there was consistently less ammonia than acid

until the sample of urine passed just after the period of tetany. The most alkaline urine had a greater ammonia than acid excretion in all experiments.

The pH of urine is uniformly increased toward the alkaline side by reason of decreased acid excretion. This may take place in the neutral bath without change of the total acid excretion, but never to the same degree. The relation of urine and blood pH is shown in table 6 in which the highest blood pH in each experiment is compared to the most alkaline urine. In three of the six experiments urine more alkaline than blood was excreted. This occurs in spite of the urine being only an average mixed sample covering the entire period during which the body temperature was being elevated, while the blood sample was taken at the very height of the change, and the difference is therefore significant.

TABLE 6

EXPERIMENT NUMBER	pH OF BLOOD	pH OF URINE
1	7.56	7.60
2	7.74	7.75
3	7.64	7.85
4	7.67	7.00
5	7.64	7.40
6	7.64	7.60

CONCLUSIONS

1. Hot baths cause a hyperpnea whose severity depends on the rate of rise of body temperature and also on the temperature level at which the rise takes place.

2. Hyperpnea involves a change in both rate and depth;—there is never a shallow respiration except in the first few moments when there is strong skin stimulation and during the recovery period.

3. pH change of blood to the alkaline side is apparently determined by the hyperpnea but is not proportional to it.

4. There may take place an excretion of urine which is more alkaline than blood, probably with varying efficiency in different individuals.

5. Oxygen does not relieve tetany, but carbon dioxide gives definite improvement.

6. On two occasions a change in the pH of the blood towards the acid side was demonstrable within a few minutes of the onset of definite tetany, and the pH change was accompanied by a fall in the total carbon dioxide content of the blood. This might readily be explained if lactic acid was produced during tetany. No evidence was obtained of any definite pH level at which tetany would occur, nor even of any critical degree of change in the pH level.

We wish to express our gratitude to Prof. H. C. Bazett not only for suggesting the problem but also for his constant interest in the experiments.

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REGENERATION OF PANCREATIC TISSUE FROM THE TRANSPLANTED PANCREATIC DUCT IN THE DOG

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Regeneration or extensive new growth of the pancreatic duct has been reported by Fisher (1924b), and regeneration of pancreas tissue (acini and islets) from the pancreatic duct tissue have been reported by several investigators, Languesse and de la Roche (1902), Cecil (1911), Bensley and Clark (1914). These observations deal with regeneration of pancreas from duct tissue left in situ, such as follows complete occlusion of the pancreatic ducts. Fisher (1924a) reported from this laboratory regeneration of a considerable mass of pancreas tissue from duct stump left in the duodenal wall in the operation of complete pancreatectomy. These two dogs were controlled by insulin. We have also reports of histological evidence of regeneration of new islets from the duct system in some cases of diabetes in man (Weichselbaum, 1908; Boyd and Robinson, 1925). But Bensley, in discussing the paper by Boyd and Robinson, points out the uncertainty in the interpretation of the histological findings in the pancreas of human diabetics.

The work here reported was undertaken at the suggestion of Doctor Carlson, on the following working hypothesis: There is experimental evidence that in normal animals the pancreatic duct can produce islets and acini, at least under some experimental conditions. Assuming an essential pancreatic factor in human diabetes, significant regeneration of islets must be absent or extremely rare, because significant recovery of carbohydrate tolerance even under insulin and dietary control is, at present, exceptional. This apparent failure of pancreas duct tissue to produce new islets may be due to *a*, congenital defect in growth potential of the pancreas; *b*, toxic injury of both islets and duct systems; or *c*, continuous injury (toxic) of the islets newly formed from the ducts. If the first hypothesis is a factor, transplantation of the pancreatic duct might be of value, provided a duct transplant takes and produces islets.

METHODS. Segments of the pancreatic duct, completely freed from pancreas tissue, were implanted under the serosa of the duodenum in dogs,

[1] This work was started in 1923 by Mr. Shaw, who completed the experiments on dogs 1 and 2. The experiments on dogs 3 to 7 were carried out by Mr. Latimer. I have checked their findings and combined their reports.—A. J. C.

at the time that complete or partial pancreatectomy was performed. The dogs were subsequently controlled by insulin, so that the urine remained nearly free from sugar. Pieces of the duct, varying in length from 1 to 2 cm., were used. The duct segments were pulled under the serosa by a cutting needle. No stitches were taken in the graft.

RESULTS. *1. Completely pancreatectomized dogs.* Dog 1. Female weighing 10.5 K. Pancreatectomy and duct transplantation, November 6, 1923.

The dog continued to lose weight for a week, reaching 8.5 kilos where she remained throughout the experiment. The urine was kept sugar-free with 25 units of insulin twice a day for the first month, 20 units twice a day for the next three months, and 25 units once a day for the last month of life. One hundred sixty-two days after operation the dog died suddenly from undetermined cause. At autopsy there was found at the site of transplant a small piece of tissue about 1 cm. in diameter. The rest of the duodenum was entirely free from pancreatic tissue. This tissue was removed, placed in Zenker's solution, sectioned and stained with hematoxylin and eosin.

Microscopically the tissue is composed of small lobules associated with lobes. The lobules are united by a delicate and relatively loose fibro-elastic connective tissue. Numerous and varying sized ducts are seen. The inter-lobular ducts are lined by a single layer of columnar cells. The intercalary ducts terminate by passing into the acinus. The acinar cells are tall and irregularly columnar or pyramidal in shape. In addition to the above there are certain larger and smaller spheroidal collections of polyhedral cells which lie in the inter-acinar connective tissue, determined to be islands of Langerhans.

Dog 2. Weight 12 kilos. Prepared as dog 1. At no time did this dog show any tolerance to sugar, needing approximately 45 units of insulin a day throughout the experiment to keep the urine sugar-free. The dog died of pneumonia 72 days after the operation. At autopsy the duodenum was free from pancreas tissue. The duct transplant was recovered and prepared for microscopic study. The main duct was lined with a single layer of high columnar cells, surrounding this main duct were numerous smaller tubules lined by a single layer of cuboidal cells which in serial section were found to be connected with this main duct. There was no definite evidence of acinar or islet tissues, but duct lived and smaller ducts had developed from the original transplant.

Dog 3. Female weighing 8 kilos. Operation June 6, 1925. All traces of glandular tissue were removed from the ligated duct stump to see whether the duct stump in situ would regenerate glandular tissue. Three implants of the main duct were made under the serosa of the jejunum. The urine was kept sugar-free by means of insulin. The wound healed rapidly, and the animal was in good condition until three days before its

death, August 3, 1925, fifty-eight days after the operation. The cause of death was pneumonia. At autopsy the transplants were found. They contained a clear fluid. At the duct stump, between the ligature and the gut, was a piece of tissue weighing 0.3 gram and grossly resembling glandular tissue. Microscopical examination of the transplant showed it to be living and to have formed a great many small tubules, but no definite acini or islets. Microscopical study of the tissue taken from the duct stump showed it to be composed partly of fibrous connective tissue and partly of newly formed pancreas tissue containing both acinar and islet formations.

2. Partial pancreatectomy. Dog 4. Male, weighing 9.4 kilos. Operation, January 17, 1925. Transplants of both the main and the accessory ducts were made under the serosa of the jejunum. The animal died of pneumonia February 9, 1925, twenty-three days after the operation. Both transplants were recovered. Microscopically they were found to be living and to have begun to proliferate new tubules. No distinctly glandular tissue was present.

Dog 5. Male, weighing 14 kilos. Operation, April 16, 1925. The operation consisted in an almost total pancreatectomy; a piece of glandular tissue about the size of a pea being left attached to the duodenum. Three implants were made of the main duct, and one of the accessory duct, all under the serosa of the duodenum and jejunum. For twelve days from eight to twenty-four units of insulin were required to keep the urine sugar-free, but after the twelfth day no insulin was required. The animal recovered rapidly from the operation, gained weight, and remained in perfect health. The dog was etherized June 30, 1925, seventy-five days after the operation. At autopsy three of the transplants were recovered. Each of the transplants was filled with a clear fluid. Microscopical study of the transplants showed that they were living, and that many small tubules had been formed, with indication of regenerating some gland cells.

Dog 6. Male, weighing 7.5 kilos. Operation, May 1, 1925. Three transplants of the main duct were made under the serosa of the ileum. The animal recovered slowly from the operation, but after recovery remained in very good health. The dog was etherized July 15, 1925, seventy-six days after the operation. At autopsy the three transplants were recovered. Each transplant was filled with a clear fluid. Microscopical study of the transplants showed them to be living and to have proliferated with the formation of many small tubules, probably with the regeneration of some glandular tissue.

Dog 7. Male, weighing 8 kilos. Operation May 15, 1925. Three transplants were made of the main duct under the serosa of the duodenum and jejunum. The animal made a rapid recovery and was in good health throughout the experiment. It was etherized July 15, 1925, sixty-one days

after the operation. At autopsy all the transplants were recovered. Each transplant was filled with a clear fluid. Microscopical examination of the transplants showed them to be living, with the formation of many small tubules, and probably with regeneration of some glandular tissue.

Discussion. The objection might be raised to these experiments that not all of the glandular tissue had been removed from the duct prior to transplantation. But in cases of six animals autopsied from six to fourteen days after the transplantation, no traces of glandular tissue was found. Therefore we feel safe in concluding that the regeneration we have observed came from the duct tissue.

Fisher (1924a, b) failed to observe any regeneration in his duct transplants, but the longest he carried his experiments was forty-two days. Since all of the transplants carried over fifty-eight days showed the formation of many small tubules and indications of regenerated glandular tissue, and since transplants carried less than fifty-eight days showed no evidence of regeneration, it is quite obvious that the time factor is very important in the regeneration of pancreatic tissue from a transplanted duct. Further, the fact that the above-mentioned time factor operated in both totally and partially depancreatectomized dogs shows that the time factor is much more important than the diabetic factor.

The presence of the fluid in the ducts (dogs 5, 6, 7) suggests that there had been a regeneration of anicar cells but that these had degenerated some time prior to the autopsy because the secretion did not find an exit. The cells of the duct are not known to have a secretory function, but we do know that on ligating the pancreatic duct the acinar cells degenerate. The regeneration of the glandular tissue from the duct stump corroborates Fisher's findings. This tissue, as in Fisher's case, had a drainage into the gut. This indicates that in the transplants regeneration of pancreas tissue occurs in all probability more rapidly and completely after the duct has established connection with the lining of the intestine.

CONCLUSION

In completely or partially pancreatectomized dogs (the diabetes being controlled by insulin) homografts of the pancreatic duct in the intestinal wall become vascularized and undergo varying degrees of differentiation or regeneration towards pancreas tissue. In one case (dog 1) a small mass of acini and islets was found regenerated from the transplant 162 days after the transplantation. In all of the experiments prolonged for two months or more regeneration of ductlets was always in evidence, with some indications of acini and islet formation. When the transplanted and regenerating duct does not establish connection with the lumen of the gut, secretion accumulates in the ducts. This factor probably retards the regeneration of pancreas tissue. Operative methods facilitating such duct-gut con-

nection would probably yield more positive results than those obtained in the present work.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF LIGHT

VII. Blood Calcium in Direct Irradiation of Blood

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In a previous publication (Reed, 1925b) were reported the effects on blood pressure in experiments in which the blood of etherized dogs was irradiated directly by a carbon arc while flowing through a quartz tube interposed in the carotid artery. In view of the recent extensive investigations on the influence of irradiation on blood calcium, it has seemed desirable to determine the calcium content of the blood in these experiments.

There have been many reports, both clinical and experimental, to the effect that irradiation with various kinds of light has produced an increase in blood calcium. Huldschinsky (1919), Hess and Gutman (1922), Kraemer, Casparis and Howland (1922), Tisdall (1922), Lesué, Turpin and Zezinc (1924), Wöringer (1924), Crowell (1925), Blum, Delaville and Van Caulaert (1925), Maynard, Goldberg and Miller (1925), Mitchell and Johnson (1925) have all reported that blood calcium was increased in cases of rickets by some form of irradiation, usually repeated on successive days. Similar results have been obtained in experimental investigations of rickets and various deficient diets (Orr, Magee and Henderson, 1924), (Hess, 1924), (Powers, Park, Shipley, McCollum and Simmonds, 1922), (Chick), (Webster and Hill, 1925).

DeGennes (1925) was able to improve blood calcium in both rickets and spasmophilia. He was of the opinion that hypocalcemia is a sign and not a cause of rickets and in old cases does not occur. Wöringer (1924) believed that the absence of light is the primary factor in rickets, but Goldblatt and Soames (1923) believed this factor secondary to some other, since irradiation is not a complete substitute for absence of certain substances in a rachitic diet. Marfan (1925) agrees with this idea. Blum, Delaville and Van Caulaert (1925) were convinced that even low mineral content of the blood plays a secondary rôle although Webster and Hill (1925) maintained that the histological picture of the bones in experimental

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animals follows closely the blood content in calcium and phosphorus, and that the primary factor was decreased absorption of these elements from the intestinal tract.

With a diet low in calcium but otherwise well balanced Maynard, Goldberg and Miller (1925) found that guinea pigs exposed to sunlight showed augmented assimilation of calcium. Orr, Magee and Henderson (1924) found increased absorption *and retention*.

In addition to augmented weight in growing rats irradiated, Lesué, Turpin and Zezinc (1924) found the total calcium content increased. Mitchell and Johnson (1925) assigned the increased calcemia in case of rickets under irradiation to increased retention.

Grant and Gates (1924) found that irradiation of normal rabbits caused hypertrophy of the parathyroids which they suggested is related to calcium metabolism. This suggestion is important in view of the findings of Casparis and Kramer (1923), Falkenheim and György (1924) and Ferri (1925) that cases of infantile tetany may be successfully treated by phototherapy, with increase in blood calcium after several days.

Marfan, Dorlencourt and Turquety (1925) reported that irradiation in severe cases of craniotabes resulted in immediate improvement and complete cure after successive treatments. Novak and Hollander (1923) and Hollander and Cottle (1925) reported that blood calcium is always low in perennial hay fever and that it is increased by irradiation.

Mayerson, Gunther and Laurens (1925) reported that in normal dogs irradiation for one hour a day for eight days *decreased* blood calcium during that time but that it returned to normal shortly afterward. But irradiation for two-hour periods increased blood calcium with a proportionate increase in urinary excretion of calcium, indicating increased absorption.

Howe and Medlar (1924) in a study of calcium and phosphorus in the blood serum of patients undergoing irradiation decided that the treatment had no effect on these constituents provided they were within the normal range already. Moritz (1925) came to the same conclusion with regard to normal rabbits.

From this evidence it appears that irradiation of any kind, including x-rays (Piccaluga, 1924), may cause an increase in blood calcium if this is already lowered. All reports of increase show this to be the case. Since Essinger and György (1924) and Lesué, and Turpin and Zezinc (1924) have reported that under no condition does short irradiation induce any change in calcium, it was predicted that under the conditions of our experiments there would be no increase in calcemia. But it has seemed advisable to investigate the point because of its importance in connection with the mode of action of light in rickets.

A sample of blood was drawn from the femoral artery immediately after anesthesia was induced. In all animals irradiated the blood was hepari-

nized intravascularly (Reed, 1925a). In control experiments this method was employed about half of the time, in others the samples were heparinized in vitro. After at least one and one-half hours a second sample was drawn. The method of calcium determination was a modified form of the Tisdall method which will be described in detail and discussed in a later paper.

The technic of irradiation has been described in the paper already referred to (Reed, 1925b). A detailed tabulation of the results obtained in control experiments is shown in the following table. Amounts are expressed as milligrams of calcium per 100 cc. of plasma.

EXPERIMENT	Ca AT BEGINNING	Ca AT END
1	13.20	14.35
2	11.88	11.48
3	11.03	11.99
4	12.09	13.33
5	10.49	11.23
6	13.16	12.44
7	11.30	11.75
8	12.00	12.00
9	12.27	10.80
10	12.00	10.03
11	10.08	13.02
12	11.30	12.00
13	13.75	11.35
14	11.65	10.90
15	10.00	10.15
16	10.15	9.35
17	10.30	
	11.58	11.63

The slightly higher figure obtained from averaging the results of analyses of the second sample might be due to slightly greater concentration of the blood at the end of the experiments, but the difference is scarcely great enough to be of any significance whatever.

The results obtained from irradiation experiments are shown on following page.

While the average amount for this series is somewhat higher than for the control series it will be noted that the normal range in individual dogs is very wide. Hence, it may be concluded from a comparison of these two series of experiments that direct irradiation, even of great intensity and over a relatively long period of time, of circulating blood does not produce any change in the blood that in any way constantly affects its calcium content.

EXPERIMENTS	MILLIGRAMS OF Ca PER 100 CC. OF PLASMA	
	Before irradiation	After irradiation
1	9.55	11.42
2	9.38	9.14
3	10.00	10.03
4	12.75	13.50
5	9.70	9.40
6	11.50	11.70
7	9.70	10.40
8	12.80	13.90
9	11.37	11.07
10	9.32	8.30
11	11.26	10.10
12	9.50	9.75
13	10.70	10.10
14	9.50	9.80
Average	12.21	12.38

One can readily accept, on the basis of the reports by various investigators, the idea that light exercises some influence on the blood since in every form of phototherapy the most pronounced effects are general rather than local. Since, under no condition, has anyone reported any alteration of calcemia until several hours after irradiation and usually only after several applications, it is not surprising that our results are negative. This then is strongly suggestive of the idea that the increased calcemia noted after phototherapy in rickets, while doubtless mediated through the blood, is due to some specific change that in some way affects absorption and retention of calcium, rather than mobilization of calcium already stored in the tissues.

It is granted that these experiments present no very strong evidence on these latter points. Further experiments will, however, be undertaken with a view to securing more definite evidence.

SUMMARY

Direct irradiation of blood in vivo with a carbon arc does not produce any immediate change in blood calcium in dogs under ether anesthesia.

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A CRITICAL STUDY OF THE EVIDENCE FOR THE PRESENCE OF AN ISOMER OF d-GLUCOSE IN BLOOD

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DISCUSSION OF PROBLEM. Very few of the changes undergone by carbohydrate during its metabolism in the animal body are known with certainty, and the initial change which occurs in the comparatively stable form of glucose that is absorbed from the alimentary tract is as perplexing as any.

Winter and Smith (1922), (1923) suggest that the first step in the metabolism of glucose is the alteration of the stable equilibrium α-β-glucose into the unstable γ-glucose. They support this view by observations upon the optical activity and reducing power of the sugar in normal and diabetic bloods. They report that protein-free extracts of the sugar in normal blood show an optical rotatory power much below that to be expected if the copper-reducing power of the extracts were due entirely to α-β-glucose. The optical rotatory power does not remain constant, but rises, according to their statements, to near the value to be expected on the basis of the copper reduction figures. Moreover, they find that diabetic blood behaves differently, the rotatory power being high at the outset, with a tendency to fall when the extract is allowed to stand for several days. Winter and Smith (1923) suggest that the high rotatory power of diabetic blood is due to the presence of di- or poly-saccharides. They observe a return of diabetic blood sugar to the normal type under insulin treatment, therefore they postulate that the function of the internal secretion of the pancreas is to convert stable α-β-glucose into the highly reactive γ-form.

The object of the present research, upon which a preliminary report has been made (Visscher, 1924), is to study the relation between the optical activity and the reducing power of blood extracts prepared in various ways, and to study the changes in those properties occurring when the extracts are allowed to stand for some time. It is hoped that in this way light will be thrown upon the source of the discrepancy between the optical rotatory and the reducing powers of the extracts, and the nature of the changes occurring when the extracts are kept for a period of time.

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EXPERIMENTAL METHODS. In the first group of experiments the experimental technique of Winter and Smith (1923) is duplicated precisely. It consisted in adding one volume each, of 10 per cent sodium tungstate, and of two-thirds normal sulphuric acid, to a volume, usually 100 cc., of blood. The precipitate is removed by filtration through a good paper, the filtrate is then concentrated to about 5 cc. by distillation "in vacuo" at 40°C. This concentrate is taken up in 85 per cent ethyl alcohol, using about one and a half times the original volume of blood. The alcohol precipitates the tungstic acid left in solution. After filtering through a heavy paper the filtrate is again concentrated "in vacuo" at 40°C., until all of the alcohol is removed and only 10 cc. of aqueous solution remain. This water solution is then studied chemically and optically. Hereafter in this paper extracts prepared in this way will be called "two-thirds normal acid extracts."

In a second group of experiments the same general procedure is followed except that normal in place of two-thirds normal acid is used in the protein precipitations. These extracts will hereafter be referred to as "normal acid extracts."

In a third group of experiments a volume of 4 per cent uranium chloride is added to the volume of blood, thus precipitating the proteins. After filtration the fluid is concentrated to about 5 cc. under reduced pressure at 40°C. This residue is taken up in 85 per cent alcohol, one and a half volumes, and filtered, the filtrate again being concentrated "in vacuo" at 40°C. The extracts so prepared will hereafter be designated as "uranium chloride extracts."

Defibrinated ox-blood was used in the majority of the experiments, but oxalated human, rabbit and dog bloods were used in a few cases. There is no essential difference in the behavior of the various bloods.

In determining the copper-reducing power of the extracts, the Folin-Wu (1919) and the Shaffer-Hartman (1921) methods for glucose estimation have been used on different occasions. The results are expressed in per cent of glucose, although, as will be pointed out later, glucose is not the only reducing substance present.

In measuring the optical rotatory power of the solution a Schmidt and Haensch three field polarimeter with a Ventzke scale is used. A reading of 1° on the Ventzke scale corresponds to a reading of 0.3460° on the circular scale for the D line of the spectrum at 20°C. The vernier of the instrument reads to tenths, so the accuracy is equal to approximately 0.03° on the circular scale. The readings can be interpolated between the vernier divisions, but the accuracy possible in judging comparative light intensities does not warrant such precision.

For the source of light a 100 watt Mazda Daylite lamp is used. The light is enclosed, and that entering the polarimeter is filtered through a saturated solution of potassium bichromate. One, two and four decimeter

polarimeter tubes have been used in different experiments, the longer tubes being used whenever the quantity of fluid would permit.

The readings are made in a dark room, and special precautions are taken to have the observer's eyes become dark-adapted by five or ten minutes' stay in the dark room before readings are made. Likewise, more than five or six readings are not taken without a period of rest, for it is found that fatigue destroys the fine accuracy which is necessary for the work.

The polarimeter readings are either given directly, or are expressed as the per cent of glucose required to bring about the same rotation. The mode of recording used is noted in each figure.

Hydrogen ion concentration determinations were made on many of the extracts prepared during this work. Both colorimetric and electrometric methods have been used, the latter the more frequently. The apparatus consists of a Leeds and Northrup type K potentiometer, a Leeds and Northrup galvanometer, a Bailey hydrogen electrode and a normal calomel electrode.

RESULTS. The experiments in the first group, namely, those in which normal blood is used and the proteins are precipitated from it by sodium tungstate and two-thirds normal sulphuric acid yield the following results.

These "two-thirds normal acid extracts" are entirely uniform in showing an initial optical rotatory power below that expected from the copper reducing power. Figures 1 and 2 give the complete record of several typical experiments, and show that the initial polarimetric value is below the copper reduction value for the sugar in the extracts.

Likewise it is uniformly found that there is a rise in the dextro-rotatory power of the extracts during the course of one or several days. The first two figures show this fact, but it is shown more clearly in figure 3, where the polarimetric readings for every experiment in the first series are recorded. In figure 3 each curve represents a complete separate experiment, and the points on the curve represent the polarimetric readings at particular times after the preparation of the extract. The time of the first reading after preparation of the extract is taken as zero.

It may be noted that the rotatory power is always low at zero time, in one case there is actually a levo-rotation. The rise is more marked in some cases than in others, but the upward trend is invariable and unmistakable.

A third fact observed in the experiments in figure 3 is the fall in rotatory power after the peak is reached. The time at which the fall begins is variable. It sometimes occurs after the first day, but occasionally not till after the fourth. The fact that it occurs is significant, however, as will be pointed out in the discussion of these results.

2. In the second group of experiments, in which a stronger acid is used in conjunction with the sodium tungstate in the protein precipitation, the results obtained are quite different. Instead of the low initial value,

rising to a maximum and falling again, these experiments are almost uniform in showing a high initial rotatory power, greater than the reducing power. Figure 4 shows the data from a typical experiment.

Of the eight experiments in this group, only one shows a tendency for the rotatory power to rise. The rotatory behavior of all these extracts is shown in figure 5. In this figure the polarimetric readings are recorded directly, as they were in figure 3, in order to permit of judgment as to the significance of changes observed in the two series of experiments. It is evident that the behavior of the "normal acid extracts" is in no way similar to that of the "two-thirds normal acid extracts" of the first series. On account of the larger amount of acid added, the hydrogen ion concentration of the "normal acid extracts" is higher than that of the two-thirds normal.

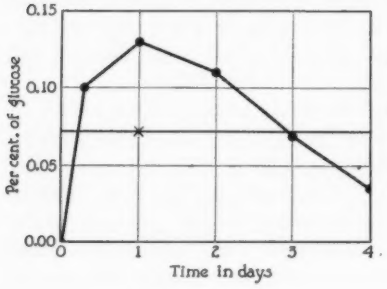

Fig. 1

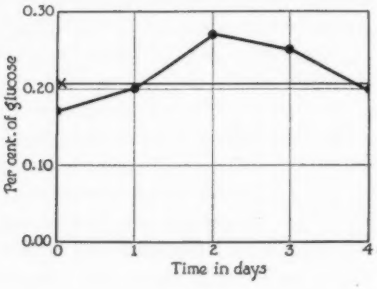

Fig. 2

Fig. 1. Changes over a period of days in the rotatory power of a "two-thirds normal acid extract" of ox blood (see text under Methods), and a comparison with the copper-reducing power.

Dots—polarimetric readings expressed as per cent of glucose. Cross—copper reduction value of the extract expressed as per cent glucose. Polarimetric readings made in a 2 dcm. tube. The hydrogen ion concentration of the extract was pH 4.5. The polarimetric value is seen to rise and then fall.

Fig. 2. Changes during several days in the rotatory power of a "two-thirds normal acid extract" of human blood, and a comparison with its copper-reducing power.

Dots—polarimetric readings made in a 1 dcm. tube and converted to per cent glucose. Cross—copper reduction value as glucose. Extract has pH 5.8. A rise and subsequent fall in polarimetric power is observed, not as extensive as in figure 1.

Hydrogen ion concentrations are given in the description of the experiments under figures 1, 2 and 4. These values are representative of those found in the two groups of experiments, the normal acid extracts having a much lower pH than the two-thirds normal extracts, just as would be expected. The significance of the difference in polarimetric behavior obtained with the two types of extracts is discussed later.

3. When uranium chloride is used as the protein precipitant the polarimetric behavior of the extracts is found, as figures 6 and 7 show, to differ

from that of the "two-thirds normal acid extracts." In four experiments the rotatory power is never found to rise during the course of time. Instead, in one experiment it falls sharply, and in the other three experiments of the series it falls very slightly. In general these extracts behave like the "normal acid extracts," by tungstic acid precipitation, certainly not like the "two-thirds normal acid extracts."

4. In order to meet the objection that fermentative or bacterial decomposition of the glucose might be responsible for the changes in rotatory power observed in these extracts, a series of control experiments was done

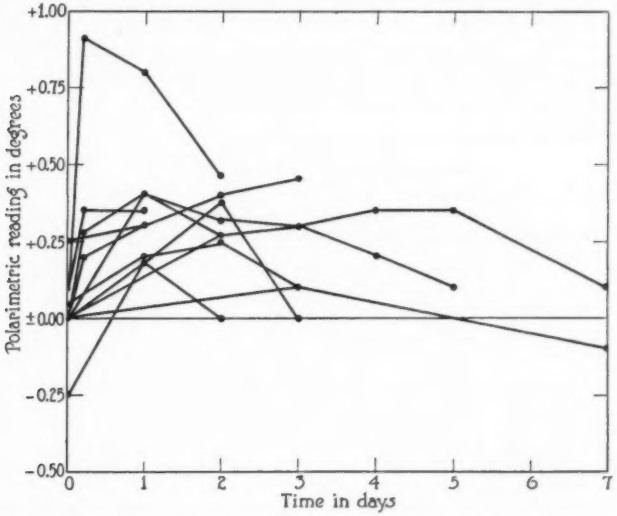

Fig. 3. Showing the polarimetric behavior of "two-thirds normal extracts" over a period of days.

Ordinates show polarimetric readings in degrees on the Ventzke scale; abscissa shows time. Each curve is a series of readings upon a single extract. This figure contains the polarimetric readings for all of the experiments with "two-thirds normal extracts." The curves are concordant in showing a gradual rise, then a fall in the rotatory power of the extracts.

to determine the changes in pure glucose solutions over periods of time. Figure 8 shows the results of a typical control experiment. A 0.5 per cent glucose solution was made slightly alkaline to bring about immediate mutarotation, it was then made slightly acid with H_2SO_4 and its rotatory power observed over a period of three days. It can be seen that there is no significant variation in the readings from day to day. That is invariably the case until eight or ten days have passed, when moulds frequently begin to develop abundantly enough to lower the rotatory power.

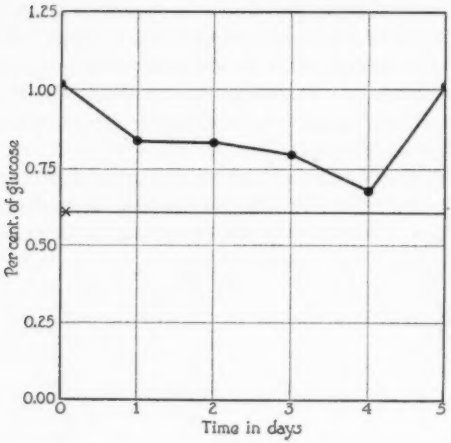

Fig. 4. Rotatory and reducing power of "normal acid extract" of ox-blood.

Dots—rotatory power expressed in per cent glucose. Cross—reducing power in per cent glucose. Extract prepared as indicated in text from ox blood. Hydrogen ion concenration of extract pH 2.5.

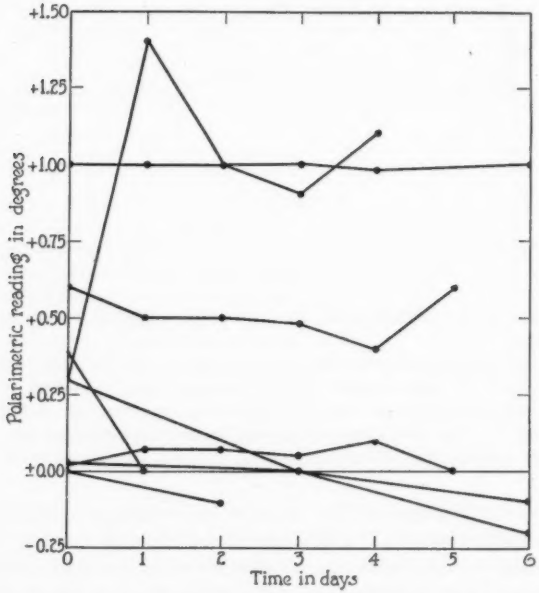

Fig. 5. Polarimetric behavior of normal acid extracts of ox and human bloods.

Dots—Direct polarimetric readings in degrees on the Ventzke scale, taken at twenty-four hour intervals. The absence of rising rotatory power, except in one conspicuous instance is significant in contrast with the uniform tendency in that direction to be observed in figure 4.

DISCUSSION OF RESULTS. A great deal of caution must be exercised in evaluating these or any other experiments upon the optical activity and reducing power of more or less completely deproteinized extracts of blood. Such extracts have been referred to by some writers, especially Winter and

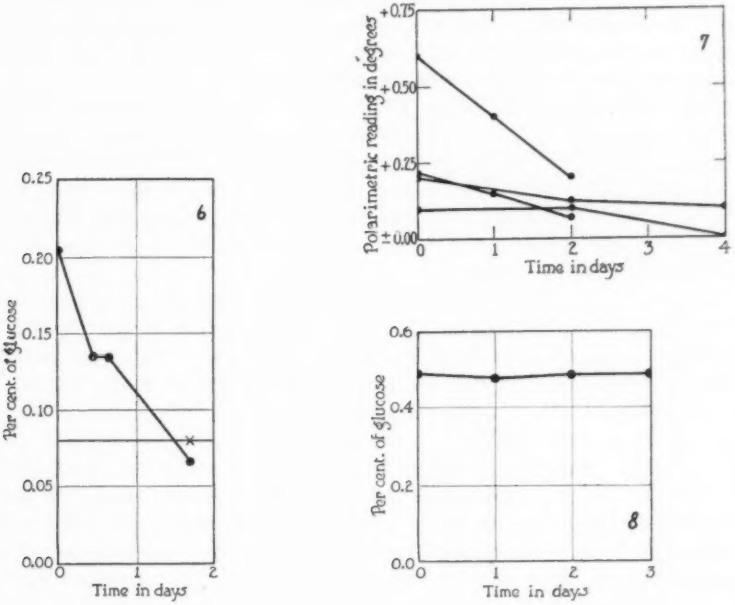

Fig. 6. Optical rotatory and reducing power of a uranium chloride extract of ox blood.

Dots—rotatory power expressed as per cent of glucose. Cross—reducing power as glucose. The rotatory power falls to about 40 per cent of its initial value on two days' standing.

Fig. 7. Optical rotatory behavior of a number of "uranium chloride extracts" of blood.

Dots—polarimetric readings in degrees on the Ventzke scale. A complete absence of any rising values is seen.

Fig. 8. Rotatory power of pure glucose in acid solution over a period of time.

Readings made in a 2 dcm tube. The experiment here recorded is one of a large number of control experiments yielding similar results.

Smith (1922), (1923), (1924) as extracts of the sugar in the blood. It is true that they contain the blood sugar, but it must be remembered that the sugar is in grossly impure form, since the extracts also retain all of the amino acids, the lactic acid, the fatty acids, and those nitrogen metabolites in the blood which are soluble in 85 per cent alcohol. To show the in-

fluence that these "impurities" in the sugar extracts exert upon their rotatory and reducing powers, a tabulation of the glucose estimated by both methods is presented. This tabulation cannot include all of the experiments done, because in about one-fourth of the experiments the reducing power was not estimated. Every experiment in which both methods of estimation were used is included in the averages. In recording the rotatory power of the extracts, the highest value obtained has in each case been taken for comparison with the reducing power.

This table is self-explanatory, and it shows clearly an excessive divergence between the two methods of estimation, too great to be due to experimental error. Winter and Smith (1922), (1923), (1924) likewise found very large discrepancies, but appear to have disregarded them as experimental error. A glance back to figure 8 will show that the experimental error is not more than 1 or 2 in the second decimal place, or less than 10 per cent of the total quantity.

It is the claim of Winter and Smith that the sugar in normal blood reverts back to α-β-glucose on standing. The extracts should therefore arrive at a stable state, in which the two methods of estimation would agree. It has been impossible in these experiments to verify either of those claims. Table 1 shows the average deviation, independent of sign, to be 61.5 per cent. Moreover the other condition, that of a stable state, is not found. Reference to figure 3 will show that under the condition of Winter and Smith's experiments the polarimetric reading never remained constant for more than two days. After the rise is completed a fall ensues promptly. This fact argues strongly against the suggestion that the rising rotatory power is an evidence of the conversion of an unstable into a stable form of glucose. If that were so one would be compelled to say that the early changes were dependent upon, while assuming that the later changes were independent of, the sugar in the extract, for it is impossible to conceive of a stable equilibrium shifting automatically to an unstable state. A more simple view is that the other constituents of extracts of blood are responsible for their peculiar behavior, since on such a view it is possible to explain the effect of other protein precipitants. There is no very evident reason why increasing the acidity during the protein precipitation, or using another method of protein precipitation should affect the behavior of the sugar in the extract, but such procedures do affect the other constituents of the extracts. It has been shown by Merrill (1924) that increasing the acidity at which the tungstic acid precipitation of proteins from blood is carried on, increases the completeness of their precipitation. There is thirty times as much nitrogen left in the filtrates when the precipitation is carried on at pH 5.8 as at pH 1. Proteins are levo-rotatory and any traces left in solution would give the low initial rotatory power observed in our first series of experiments, and in those of Winter and Smith.

The more complete precipitation of protein in higher acid concentration would explain the absence of a low rotatory power in our second series of experiments. Working on glucose in contact with intestinal mucosa, Stiven and Reid (1923) found that there was disagreement between copper reduction and polarimetric values for the sugar only when the solutions were contaminated by protein from the intestinal walls. Denis and Hume (1924) have attempted to corroborate Winter and Smith's observations but have been unable to do so. They made a change in their procedure

TABLE 1

Showing the percentage difference between the copper-reducing power and the maximum rotatory power of the protein-free extracts used in these studies

The average deviation was found to be 61.5 per cent. The number of deviations of more than 25 per cent is 14, or 82 per cent of the total number.

EXPERIMENT NUMBER	COPPER REDUCTION ESTIMATION IN PER CENT GLUCOSE	POLARIMETRIC ESTIMATION IN PER CENT GLUCOSE	POLARIMETRIC VALUE EXPRESSED AS PER CENT OF COPPER REDUCTION VALUE
	per cent	*per cent*	*per cent*
1	0.15	0.125	84
2	0.06	0.10	166
3	0.09	0.12	133
4	0.12	0.17	141
5	0.12	0.26	225
10	0.22	0.13	60
15	0.076	0.034	45
16	0.073	0.136	189
18	0.24	0.48	200
20	0.061	0.102	166
21	0.665	0.68	103
22	0.20	0.17	85
23	0.08	0.204	250
100	0.204	0.272	133
101	0.188	0.238	126
102	0.224	0.450	200
111	0.069	0.13	189

for extracting the sugar, which makes their experiments not quite comparable to those of Winter and Smith, or to those of the first series reported here. They employed aluminium hydroxide to clear the extracts for polarimetric reading. By adsorbing any remaining protein they were thus able to eliminate the peculiar polarimetric behavior which appears to be related to the protein content of the extracts.

A number of years ago Griesbach and Strassner (1913), and later Stepp (1919), (1922) showed that when proteins and other interfering substances were very carefully removed from blood extracts one could obtain very good agreement between polarimetric and copper reduction estimations. Later findings seem to point in the same direction.

It is of interest, although not explicable, that Winter and Smith found diabetic blood extracts to behave as did "normal acid extracts" in this work. The fact that the behavior of extracts of diabetic blood could be duplicated with extracts of normal blood indicates that the distinction is more artificial than fundamental.

SUMMARY

1. The optical rotatory power of extracts of blood, deproteinized by the method outlined by Winter and Smith, is not constant, but rises more or less rapidly for several days and then falls gradually.

2. Altering the method or conditions of the protein precipitation changes the behavior of the extract of blood.

3. The disagreement between the maximum polarimetric and the copper reduction values of the extracts indicates strongly that substances other than glucose are influencing those values.

4. Present evidence does not support the conception that the sugar in blood is the unstable γ-glucose.

I wish to express my gratitude to Prof. Frederick H. Scott, and to Dean Elias P. Lyon for providing the facilities for prosecuting this and other researches, and for much valuable advice and criticism.

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STUDIES OF THE THYROID APPARATUS

XXIX. The Rôle of the Thyroid Apparatus in Growth

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About six years ago a series of experiments was begun, the purpose of which was to determine the relative importance of the thyroid apparatus in the growth of the mammal at different stages of development. The investigation is part of the general plan for the study of growth, and more particularly of the rôle of the incretory organs therein. During the interval from the beginning to the completion of the accumulation of the data, a number of reports have been published on the growth of the body as a whole and of its parts after the removal of the thyroid apparatus and the parathyroid glands at 75 and 100 days of age. Since these papers give the conditions of control, the methods of procedure and analysis, and the criteria used in determining the success of the operative measures a repetition is unnecessary. It might be noted that the albino rat (Mus norvegicus albinus) was chosen as the animal representative of the group because more is known of its growth than of any other, and because there was available adequate material of known history under the conditions of control essential for such an undertaking.

Now that the data are collected it becomes possible to present a coherent panorama of the results and from their analysis an interpretation.

The analysis of the course of growth in body weight subsequent to the initiation of the glandular deficiencies has brought out facts of such importance to the interpretation of the results as a whole that it seems wise to confine the present paper to this phase of the problem, rather than to include the growth in body length and tail length as was done in the earlier reports (1), (2).

Table 1 gives the distribution of the animals from which the data to be presented in this and succeeding papers were obtained. The sum of the figures does not represent the number of animals used, since it often happened that an unoperated rat served as control for its thyroparathyroidectomized, parathyroidectomized and operated control litter mate of the same sex. On the other hand more than the recorded numbers were operated upon; but they were not suited for this phase of the study because of in-

complete removal of the glandular structures, or because they died before they were 150 days of age.

It should be noted that in several but not all age groups there were two or three thyro-parathyroidectomized rats which exhibited a growth retardation so much greater in degree and different in character from that of the group as a whole as to justify their exclusion on the basis of abnormal reaction. These will be treated separately. In order to save space, no details with respect to the operated controls will be given. These are on file at the Wistar Institute and are available for inspection. They show that the operative procedure without glandular extirpation produces no significant alteration of growth from that of the unoperated controls.

TABLE 1

The distribution of the rats used in this analysis

GROUPS		AGE SERIES*											
		23		30		50		65		75		100	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Thypars	Tests	11	10	10	13	8	10	13	11	12	12	12	13
	Controls	9	9	8	10	8	7	10	9	10	10	11	11
Parathypars	Tests	12	12	16	13	12	15	14	15	13	10	14	15
	Controls	11	10	13	12	9	12	10	10	9	8	11	11
Operated controls	Tests	5	3	5	6	5	5	4	6	6	5	5	2
	Controls	5	3	5	6	5	5	4	6	6	5	5	2
Reference	Controls	20	20	20	20	10	10	10	10	10	10	11	10

* Age at which glandular deficiencies were initiated and reference controls dissected for basic data.

The small size of the rats at 23 and 30 days of age necessitated the use of ten pairs, or twenty rats, of each sex of each age, for the organ weights of the reference controls.

As stated in another place (1), weekly records were made of the individual body weights. The average values of the several groups are given in table 2. They make possible a comparative analysis of the course of growth as distinct from that of the degree of total retardation. When the raw values are plotted on ordinary cross-section paper certain trends appear. I shall not give the twenty-four charts necessary for the complete presentation of the curves, since the amount of space required is more than their intrinsic value would warrant. The principal points can be observed on the two charts given. Figure 1 shows the curves of the mean weights

TABLE 2

The mean observed body weights of the several groups and series

AGE	MALE				FEMALE			
	Thypars		Parathys		Thypars		Parathys	
	Controls	Tests	Controls	Tests	Controls	Tests	Controls	Tests
The twenty-three-day-old series								
23	30	30	28	27	27	26	31	30
30	46	39	40	32	41	37	47	36
37	61	50	60	43	61	51	70	50
44	86	67	81	54	82	64	92	64
51	106	79	103	66	96	74	107	75
58	128	93	126	78	110	82	118	83
65	143	105	142	88	124	90	130	92
72	161	114	161	97	134	96	139	101
79	179	128	179	107	142	101	147	109
86	193	139	195	116	150	107	155	113
93	207	147	210	126	157	111	161	118
100	218	154	220	133	163	114	166	124
107	226	161	229	140	167	117	172	127
114	232	167	237	148	172	120	178	130
121	242	174	245	155	175	123	181	133
128	252	179	255	160	182	126	185	136
135	256	182	258	167	184	128	189	140
142	264	188	266	174	187	131	193	140
150	271	193	275	182	193	136	195	142
The thirty-day old series								
30	40	40	42	43	37	36	40	39
37	58	51	60	54	60	51	63	53
44	80	66	85	68	77	60	84	63
51	100	78	103	83	95	69	104	76
58	120	89	123	96	111	76	118	91
65	145	103	149	110	122	80	130	105
72	163	112	170	123	133	85	139	117
79	180	124	188	134	141	90	146	123
86	194	133	204	144	150	93	155	128
93	206	137	216	153	156	98	161	133
100	218	141	228	158	161	100	165	136
107	225	147	238	165	167	104	170	142
114	235	153	247	175	170	107	174	143
121	243	154	257	183	176	110	178	147
128	247	158	261	186	181	114	183	151
135	257	167	270	193	185	116	185	153
142	258	169	272	197	188	119	187	151
150	264	170	282	203	194	123	190	160

TABLE 2—*Continued*

AGE	MALE				FEMALE			
	Thypars		Parathys		Thypars		Parathys	
	Controls	Tests	Controls	Tests	Controls	Tests	Controls	Tests
The fifty-day-old series								
50	78	81	78	78	76	74	80	77
56	90	82	95	82	92	76	99	83
63	110	97	116	95	108	79	116	94
70	128	107	133	105	123	83	127	104
77	145	118	154	122	130	87	135	113
84	158	124	169	131	137	91	144	118
91	174	135	183	140	143	97	149	119
98	186	141	192	146	146	99	153	121
105	204	150	209	159	152	105	157	122
112	217	155	223	168	156	107	161	123
119	226	160	231	173	160	110	165	124
126	236	167	239	182	163	113	169	126
133	244	168	246	182	167	115	172	126
140	248	173	251	185	168	115	173	129
147	251	172	256	185	171	115	175	133
150	255	175	259	184	172	116	177	136
The sixty-five-day-old series								
65	112	120	122	123	107	100	107	101
73	140	122	142	125	118	103	124	104
80	157	132	159	135	129	107	133	113
87	174	142	177	150	137	111	144	122
94	187	147	194	156	144	115	147	128
101	200	151	208	160	151	119	154	134
108	213	158	223	162	157	122	160	136
115	231	161	241	166	160	124	164	136
122	237	165	249	170	164	127	167	136
129	247	172	260	171	170	129	174	137
136	252	172	267	169	172	132	175	139
143	261	174	275	167	177	135	182	138
150	267	176	282	169	181	138	183	140
The seventy-five-day-old series								
75	134	137	140	132	117	113	110	114
82	156	139	160	122	133	112	120	109
89	169	147	176	126	136	110	127	112
96	182	152	189	133	144	112	134	117
103	194	157	202	140	148	112	141	120
110	204	160	214	145	154	117	144	123
117	213	166	226	148	160	121	149	129
124	219	168	232	151	163	120	153	135

TABLE 2—*Concluded*

AGE	MALE				FEMALE			
	Thypars		Parathys		Thypars		Parathys	
	Controls	Tests	Controls	Tests	Controls	Tests	Controls	Tests
The seventy-five-day-old series—Continued								
131	228	174	240	150	166	122	159	135
138	234	176	247	148	172	123	162	136
145	241	179	255	148	175	123	165	134
150	246	183	257	147	179	123	169	133
The one-hundred-day-old series								
100	164	160	164	157	142	141	142	137
107	182	160	182	151	152	133	152	134
114	191	161	191	158	159	132	159	134
121	200	163	200	162	162	131	162	135
128	207	166	207	167	167	129	167	141
135	215	171	215	178	169	129	169	141
142	222	175	222	182	173	129	173	145
150	230	180	230	193	177	131	177	144

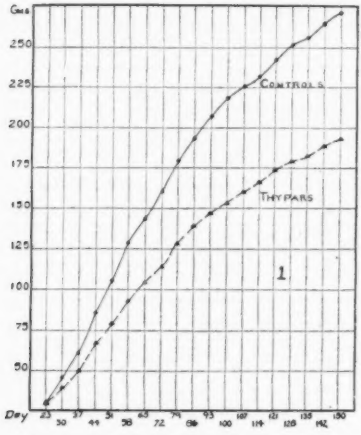
on age of the male rats thyro-parathyroidectomized at 23 days of age and their controls; and figure 2 the same data for the males operated at 100 days. The figures as a whole show that:

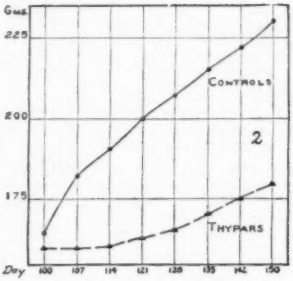
1. Growth in body weight after thyro-parathyroidectomy and parathyroidectomy at any of the stated ages (23, 30, 50, 65, 75 and 100 days) is immediately less vigorous than normal. This condition is permanent for the periods of observation.

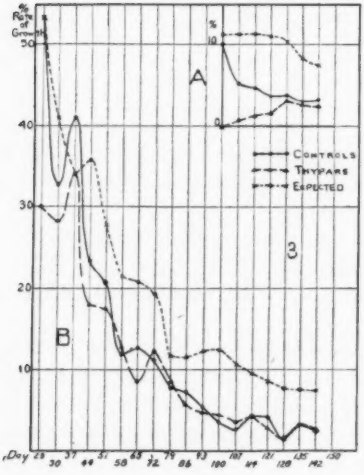
2. The deviation of the course of growth of the test groups from their controls increases with increase in the age of the animals at the time of initiation of the glandular deficiencies. The trend is definite in the thyro-parathyroidectomized groups of both sexes, though interrupted at puberty (65 to 75 days), and evident, though less regular, in the parathyroidectomized groups.

3. The lesser deviation in the younger groups is not a matter of more time for recovery. It is a matter of their greater resistance to growth retardation at the time of operation. Evidence for this is given by the fact that the older test groups show a greater deviation from their controls than do the younger during the first few weeks after glandular removal.

4. In general the deviation of the course of growth from the normal, during the first week after operation, is greater in the parathyroidectomized than in the thyro-parathyroidectomized groups. The cause of this will be discussed later.







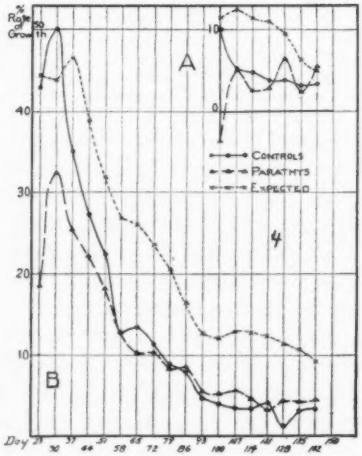


Fig. 1. Growth curve of the male rats thyro-parathyroidectomized at 23 days of age, together with that of their controls.

Fig. 2. Growth curve of the male rats thyro-parathyroidectomized at 100 days of age, together with that of their controls.

Fig. 3. A. The percentage rate of growth from week to week of the male rats thyro-parathyroidectomized at 100 days of age together with that of their controls. B. The same for the male rats operated at 23 days of age.

Fig. 4. A. The percentage rate of growth from week to week of the male rats parathyroidectomized at 100 days of age together with that of their controls. B. The same for the male rats operated at 23 days of age.

An inspection of the curves plotted from the raw data and the curves plotted from the week to week growth capacity values, fails to show any consistent qualitative differences, other than the one just mentioned, which would allow a distinction to be drawn between the result of a thyroid-parathyroid deficiency and that of a parathyroid deficiency alone. The quantitative differences, while fairly consistent, are not large. From this the conclusion would seem necessary that the effects on growth in body weight of the combined removal of the thyroid and parathyroid glands are to be assigned to the parathyroid deficiency alone. Such a view, sweeping away as it does all consideration of the thyroid as a factor in body growth, is a view which is inconsistent with what is known of the respective physiological rôles of the two organs.

The evidence justifies the belief that parathyroid deficiency produces a toxemia. The retardation of growth under these conditions can be attributed, in large part, to an inadequate nutritive level within the organism, as will be shown later.

Growth retardation in conditions of thyroid deficiency on the other hand can be primarily attributed to the lower metabolic rate produced by the glandular defect.

The conclusion would, therefore, seem necessary that the growth retardation in a condition of combined thyroid-parathyroid deficiency is the resultant of two sets of forces. But—gross expression of the toxemia of parathyroid deficiency such as is shown by defective teeth (3), hypertrophied submaxillary glands (4), cyclical character of growth (1), and marked change in calcium, magnesium and phosphorus percentage of the ash of the bones (5), does not obtain when the thyroid is removed along with the parathyroids. It is, therefore, probable that the lower metabolic level due to thyroid deficiency keeps the rate of production of the toxic substances due to parathyroid deficiency down to the point where their characteristic influence is obliterated. If this assumption is correct, it is just to interpret the sequelae of thyro-parathyroidectomy in terms of thyroid, rather than in terms of parathyroid or combined thyroid-parathyroid deficiency. While the interpretations will be based on this assumption, it is realized that the separation is probably less clean-cut than the term implies.

Proceeding to the analysis of the rôle of the thyroid in growth I will use as material for interpretation the curves of the weekly growth capacity values of the various groups of rats subsequent to the initiation of the glandular deficiency. Figure 3 gives these curves for the male rats thyro-parathyroidectomized at 23 days of age and their controls, and the same data for the male rats operated at 100 days. The values on which these and other similar charts are based are calculated from the data given in table 2, according to the method described in an earlier paper (1).

Since the percentage rate of growth (growth capacity) of the normal albino rat decreases with age it is obvious that the curve of the controls falls away from its initial high point. When the thyroid apparatus is removed, there occurs an immediate drop in the percentage rate of growth, so that the curves for the tests fall below those of the control groups. This means that less than the normal amount of new substance is being added per unit mass per unit time. After a few weeks, however, the curve of the tests comes to lie practically on that of the controls. This means that at this time the percentage rate of growth of the thyroidless animals is practically the same as that of their controls. At this time the tests are absolutely lighter than their controls because the absolute increment during the interval between the time of operation and the point of recovery has been less than normal. It is, therefore, plain that even though the percentage rate of growth becomes consistent with the value characteristic of the age of the animal, the absolute rate remains less than that to be expected from the size or age.

These facts allow the separation of the course of growth into two periods, the first being that when the percentage rate of growth of the tests is less than that of the controls: the second when it is practically the same. The discussion of the second period will lay the ground for the interpretation of the first.

It is to be noted that the duration of the second period decreases with the age of the animal at the time of glandular removal. This is because the length of the first period is practically the same in all age series, while the total period of observation is naturally shorter the older the animal at the beginning of the test.

What is the explanation of the fact that the percentage rate of growth of the thyroidless animals is normal for the age, while the absolute rate of growth is less not only for size but also for age?

Of the foodstuffs ingested by the growing organism a part is used for growth and a part for maintenance (activity). There is therefore less energy expended than ingested. Growth is the expression of the difference. The absolute amount of the difference is the capacity factor of growth for the organism and its index is the absolute rate of growth. The amount of the difference relative to total mass of the organism is the intensity factor of growth and its index is the percentage rate of growth. The absolute amount depends upon the amount of materials available for growth, which is dependent on the processes concerned in maintenance;—which in turn are regulated by the mass of active tissue and its degree of activity. There are thus two distinct processes concerned in growth, either one of which is capable of disturbance by an experimental procedure.

It is known from observation and the analyses of Robertson (6) that growth is the resultant of the action of a group of catalysts on living ma-

terial. Confining ourselves to those produced within the body it is conceivable that certain types of catalysts would exert their influence through their participation in the regulation of the processes of maintenance (or total amount of materials worked over in a given period, or capacity factor of growth): and that others would be influential in the determination of the relative increment or intensity factor. Now since in the progressive utilization of materials in building up new tissue there results a progressive increase in the amount of inactive mass as compared with the active mass, a progressive retardation of the effectiveness of the catalysts concerned in the latter process occurs. Hence the percentage rate of growth decreases with age. This inhibition of expression occurs even though the processes of maintenance still provide materials suitable for growth.

The investigations of Kendall (7), Boothby and Sandiford (8), Plummer and Boothby (9), DuBois (10) and others, show that the thyroid produces a catalyst which is a participant in the regulation of the metabolic rate, and that a deficiency in this catalyst is attended by a reduction in the metabolic or maintenance level.

From this and what has gone before it is clear that the reduction in the absolute rate of growth in the thyroidless rats below the level normal for the age and size, is due to a decrease in the absolute amount of materials worked over because of the lowering of the metabolic level caused by the thyroid deficiency.

On the other hand, although the slowing of the maintenance processes has apparently disturbed the relative amount of increment, this effect is but temporary since the percentage rate of growth, after a short period, becomes and continues to be practically that characteristic for the stage of development. This shows that the progressive change in the intensity factor with age continues its normal course in conditions of thyroid deficiency. Hence it is evident that catalysts other than those produced by the thyroid are chiefly concerned in this relation. From which it can be concluded that the rôle of the thyroid in growth is almost exclusively a matter of its participation in the determination of the metabolic level (*i.e.*, of the absolute amount of materials worked over). In this way it acts as a determinant of the capacity factor of growth.

When the percentage rate of growth of the thyroidless rats is considered from the point of view of weight instead of from the point of view of age or stage of development, it is found that the values are less than the expected.[1] This is shown on figure 3 by the difference between the dashed line which represents the observed and the dotted line which represents the expected values. That is to say the intensity of growth is less than that to be expected for animals of the recorded weight. This is apparently a con-

[1] The "expected" values were calculated from the observed weights of the controls, rather than from Donaldson's tables (11).

tradiction of what has gone before. But it is not really so, because these smaller thyroidless rats are obviously older than intact animals of the same weight would be. They are smaller because they have been growing from the time of the initiation of the thyroid deficiency by lesser increments of mass per unit time. It therefore takes them a longer time to reach the weight of the controls at any given age. They are older than the controls when they reach the same weight. Being older than intact animals of the same size they naturally have the percentage rate of growth characteristic of the age, since, as has been shown, the decrease in the strength of the intensity factor which occurs with age follows its natural course (after a period of adjustment) regardless of the lower metabolic level. Hence at any given age the percentage rate of growth of the smaller thyroidless rats must of necessity be less than that to be expected for intact animals of the same size but younger age. The phenomenon is therefore a confirmation instead of a contradiction.

A few preliminary considerations are necessary before going on to the discussion of the significance of the first period—the period of adjustment following the initiation of the glandular deficiencies—during which the percentage rate of growth is below the normal value.

The total increment in mass, or growth in weight, of a multi-cellular organism is the sum of the increment due to the increase in the number of cells, and the increment due to their increase in size and density. Included in the latter are the special cases such as the bones, where the characteristic products of cell activity are laid down outside the cell. While these two processes are hardly additive in expression, the distinction is convenient for analysis.

Although no data are available from which there can be determined the relative value of each of these factors in the total growth at any given period, or the change in ratio which occurs with age, the existence of a change, and its direction, is indicated from several sources. Histological studies have shown that the rate of increase in cell number, or mitosis, decreases with age. Since growth in mass is a process involving a catalyst, it must be subject to the laws regulating catalytic effectiveness, and hence progressively retarded by the gradual accumulation of the products of the catalyzed reactions. It is probable that the retardation is greater and comes on earlier in growth by increase in cell number than in growth by increase in cell mass. This is certainly the case in the nervous system where mitosis stops long before increase in cell size (11).

It is consistent to assume that as the animal ages, growth by increase in cell number becomes, relative to total growth, progressively less important; while growth by increase in cell mass becomes progressively more important.

Robertson (6) has demonstrated that growth by increase in cell number

is auto-catalyzed, and that its retardation can be attributed "to the progressive accumulation of auto-catalyst in the pericellular fluid." The production of auto-catalyst is the common property of all living cells of the organism. In addition to this type of catalyst, there are other stimulants to growth which are the specific products of special organs. Their influence upon growth may or may not be representable by the auto-catalytic type of curve. The thyroid produces one of these specific types of catalysts. As previously demonstrated its action in growth is a matter of direct regulation of the level of general metabolism. This is in line with Kendall's (6) statement that the curve representing this action is a straight line: that is, the increase in energy production with increasing amount of catalyst is an additive one. Further support is given by the fact that the correlation between thyroid weight and body weight is positive and linear within the usual range of thyroid activity (12).

On the other hand Torrey, Riddle and Brodie (13) have shown that thyroxin accelerates certain metabolic processes in unicellular organisms (Paramecium), but does not stimulate cell division, in fact actually retards the process. The experiments of Budington (14) are not inconsistent with these findings. Apparently contrary thereto are the reports of Ebeling (15), Champy (16), Torrey and Hɔrning (17) and Speidel (18) which indicate that thyroid gland substance and hyperthyroidism stimulate cell division. I will leave the explanation of these diversities to the workers in this particular branch of the problem and the discussion of the influence of hyperthyroidism on growth in weight to a later paragraph.

The evidence given is justification for the belief that the thyroid catalyst is largely concerned in growth by increase in cell mass rather than in growth by increase in cell number. The two are, of course, interdependent, whether thyroid function is present or lacking.

A further distinction and evidence that the auto-catalytic type of growth is not dependent on thyroid function is shown by the fact that growth remains auto-catalytic in character even though thyroid activity is lacking. This can be seen on figure 1.

Total growth in weight is the sum of the increments in active and inactive mass. In the growing animal the proportion of active to total mass decreases with age. The rate of growth in active mass is, therefore, less than that in total: and the rate of growth in inactive mass is greater than that in total. The phenomenon is factored by the decrease in growth by increase in cell number.

The reduction below the normal for the age of the percentage rate of growth which occurs upon the removal of the thyroid apparatus indicates a disturbance of a preëxisting equilibrated process. Recovery from a dislocation of equilibrium either takes the path by which a new equilibrium is established or the path by which the normal level is regained. The fact

that the percentage rate of growth later becomes and continues to be the value characteristic of the stage of development shows that the adjustment is back to the normal and not down to a new level. An analysis of the expression and mode of adjustment is indicated.

The expression differs according to the age at which the disturbance is initiated. It is as follows:

The removal of the thyroid apparatus at 23 days of age causes an immediate drop in the percentage rate of growth below the normal (fig. 3). The subsequent decrease with age is less in the tests than in their controls with the result that the curve of the former falls away less rapidly than that of the latter and the two meet, from which point they continue on together.

In the 100 day old series on the other hand (fig. 4) the loss of thyroid function has cut the percentage rate of growth to a point well below the lowest level reached by the controls, and hence the curve of adjustment rises from its initial low point to meet that of the intact animals.

The transition point between these two types of adjustment expression occurs at the beginning of the pubertal period (50 days of age). This will be discussed shortly.

At the moment of thyroid removal the ratio between active and inactive mass: the intensity factor of growth: the ratio between growth by increase in cell number and growth by increase in cell mass: the metabolic level and the concentration of catalyst of extra-thyroid origin is presumably the same in the tests as in the controls.

Immediately following thyroid removal the reduction in the metabolic level causes a decrease in the amount of materials worked over in a given time. Hence a lesser amount of new mass is added in unit time per unit initial mass and the percentage rate of growth of the tests becomes less than that of their controls. It becomes a value characteristic of an older animal. The underlying conditions are, however, different. In the thyroidless rats the proportion of active to inactive tissue is greater than in the older intact animals of the same percentage rate of growth. Other relations are obviously also different. Until the equilibrium characteristic of the stage of development is reëstablished there can be no coincidence of the percentage rate of growth.

Note should be made of the possibility that the ability of the tissues to respond to stimulation is decreased. That this is probable in connection with dietary stimulants of metabolism in conditions of thyroid deficiency can be inferred from the recent report of Baumann and Hunt (19) which showed that "complete thyroidectomy caused a gradual decrease and final disappearance of specific dynamic action."

Moreover it is possible that the nutrient level is reduced through a diminution of the scope of activity of the organs concerned in the digestion, preparation and transport of food-stuffs (20).

The lowering of the metabolic level retards the subsequent production of catalysts of extra-thyroid origin. But it, at the same time, removes an inhibition to their full expression, in that the age accumulation of the products of the growth reaction catalyzed by the thyroid is stopped. The removal of the inhibition results in a subsequent relative enhancement in their effectiveness. A slight spurt in growth by increase in cell number as compared with growth by increase in cell mass obtains. As a result the proportion of total mass represented by active tissue, after a given period, is slightly greater than the normal for the age, while that represented by inactive mass is slightly less. The catalyst concentration becomes less than normal for the age in proportion to the active mass and greater than normal in proportion to the inactive mass. As a result the superiority of the increment in inactive mass over active mass is greater than normal. As growth proceeds this naturally brings the relation between active and inactive mass to the same value as that of an intact animal of the same age. The same happens to the catalyst concentration in terms of inactive mass, and catalyst production in terms of active mass. These changes are consistent with the reduction in the amounts of material worked over due to the lower metabolic level. They can be conceived of as taking place without there having been any change in the strength of the intensity factor, other than that due to age (its full expression has, however, been prevented for the reasons given). At this point, equilibrium between the various factors having been established the percentage rate of growth becomes and continues to be that characteristic of an intact animal of the same age but heavier body weight.

This interpretation of the mechanism of adjustment is consistent with the observed facts.

The change in type of expression at puberty from a falling to a rising curve of percentage rate of growth, such as is shown by the thyroidless rats of the 100 day old series on figure 3, is due to a change in degree and not in nature of underlying response. It indicates a greater enhancement in effectiveness and production of extra-thyroid catalysts of growth, relative to the state immediately preceding the initiation of the glandular deficiency, in the older than in the younger animals. The enhancement is sufficiently marked to not only resist the retardative influence of age on growth, but is of such degree as to bring about a temporary slight acceleration in the percentage rate of growth. This greater degree of enhancement is due to the change with age in the relative proportion in total growth of growth as conditioned by catalysts other than those produced by the thyroid, and growth as determined by the thyroid catalyst.

It should be clear from what has gone before that full expression of the influence on growth of the extra-thyroid catalysts is increasingly checked by the progressive increase in thyroid participation in growth with age.

When the non-thyroid catalysts are dominant as in the younger animals the removal of the thyroid influence of relatively lesser importance in total growth, naturally results in only a slight enhancement of their effectiveness since they are closer to full expression at this time. When on the other hand the thyroid influence is dominant, as in the older animals, its removal is followed by a greater relative enhancement of the effectiveness of the extra-thyroid catalysts, since they are, preceding the removal, less fully expressed than at the earlier stage of development.

That the influence of the thyroid increases with age is shown by the fact that in the older rats growth is actually stopped for a short time immediately following thyroid removal but is not so affected in the younger (figs. 1 and 2). The temporary absolute inhibition shows that normal growth at this time is practically entirely a matter of growth by increase in cell mass as factored by thyroid regulation of the metabolic rate.

Two other phenomena exhibited during the period of adjustment are worthy of note.

The first is that neither sex nor the age at which the thyroid apparatus is removed have any consistent effect upon the duration of the period of adjustment. The shifting values from group to group make any attempt at interpretation unprofitable.

The second is that there is a progressive increase with increasing age at the time of glandular removal in the degree to which the average of the weekly percentage rates of growth of the tests is reduced from that of their controls. That is to say, the older the animals the greater the reduction in the percentage rate of growth. Indication of this was noted in an earlier paper (2). The tentative interpretation was that the reaction was due to the greater inherent growth capacity of the younger animals; the term growth "capacity" having been used as synonymous with "percentage rate of growth." The analysis given in this paper shows that the use of this terminology is inexact unless data on the whole course of growth are available.

From what has gone before it is evident that the phenomenon can be attributed to the increasing dominance of growth in cell mass (as conditioned by thyroid participation in the regulation of metabolic rate) over the growth by increase in cell number (as conditioned by catalysts other than those produced by the thyroid). For the loss of the thyroid catalyst at the younger stages when its influence is proportionately less, would produce a lesser degree of retardation than its removal at the older stages when the effectiveness of the independent factors is proportionately reduced.

Before going on to the discussion of the influence of parathyroid deficiency on growth in body weight, brief mention will be made of puberty as a factor in the determination of the response to thyroid deficiency; and also of the rôle of hyperthyroidism in growth retardation.

It has already been stated that the transition point between the two types of expression of adjustment to thyroid deficiency in the percentage rate of growth, occurs in the rats of the 50 day old series,—at the edge of puberty. A similar phenomenon is exhibited by the raw growth curves, for it is first to be seen in the rats operated at 50 days of age that growth is practically stopped for a short period immediately succeeding the glandular removal. This initial reaction is consistently shown in the older series (65, 75 and 100 days). It is not shown in the younger ones (23 and 30 days). It is at puberty that the age series shows an abrupt drop in value (of the weekly average percentage rate of growth for the period of adjustment) of the tests in terms of their controls. The question naturally arises: is the pubertal disturbance an indication of a specific enhancement of thyroid participation in growth (due to stimulation by incretory products originating in the gonads) over and above the progressive increase which occurs with age, as already discussed? The fact of the frequent occurrence of thyroid enlargement at puberty in man certainly indicates the necessity of considering the possibility seriously. Moreover a statistical study of inter-glandular weight relations, a preliminary report of which has appeared in another place (21) has shown that there is a relation between thyroid and gonads, which is, however, mediated by adrenals. Nevertheless, since the study of the growth of the parathyroidless groups shows that another interpretation must be given, the complete discussion of the point will be reserved for a later paragraph.

Attention should be called in passing to the fact that puberty brings about the same tendency to equilibration and stabilization in the percentage rate of growth of the thyroidless animals in body weight (fig. 3) as it was shown to do in the case of the bones both for weight and length and chemical differentiation (22), (23). The same phenomenon is shown in the parathyroidless rats (fig. 4). It indicates that the pubertal adjustment has a profound influence on the growth processes of the body even under conditions of disturbed metabolism.

The growth response of an organism to a condition of hyperthyroidism is not necessarily a subject pertinent to the present paper, since the experimentation on which this study was based was opposite in character. Nevertheless a brief analysis is not out of place, for the premises on which the interpretation is founded must be applicable in both types of investigation if they are well taken. It is hardly necessary to go into a detailed explanation of the mechanism of the growth retardation induced by hyperthyroidism.

The retardation in weight which follows the administration of larger amounts of thyroid substance or products to growing animals is partly a response to an increase in the general metabolic rate of such degree that the ability of the digestive system to prepare foodstuffs for assimilation in

sufficient amount to produce normal growth is exceeded. This is shown by the fact that if the dosage is pushed far enough, weight is actually lost instead of gained.

A further factor in the determination of the slower growth lies on the hypothesis of Robertson (6) of the retardative effect of auto-catalyst accumulation, and the idea developed in earlier paragraphs of the retardative effect of the increasing importance with age of thyroid participation in the growth processes. The enhancement of the metabolic rate due to hyperthyroidisim would result in an increase in auto-catalyst production so that its concentration would be that characteristic of an older animal, and hence the retardative effect stipulated by Robertson would be the value consistent with that stage of development. In addition the administration of thyroid products would result in an artificial induction of a thyroid participation in growth processes relatively greater than that characteristic for the particular stage of development at which it was initiated,—a relative participation which would be that characteristic of an older animal. The combination of these two retardative influences with the factor first mentioned would produce the observed results.

The tables of Cameron and Carmichael (24) of the growth of rats fed thyroid products, indicate in several cases a primary stimulation such as might be expected to temporarily follow an increased production of autocatalyst. This could of course be also explained as a consequence of the increase in metabolic level up to but not beyond the capacity of the digestive system to provide metabolitus in adequate amounts, prior to the attainment of complete effectiveness of the particular dosage employed. I believe, however, that the growth retardation which occurs in artificially induced hypothyroidism is the expression of the interaction of the three factors described.

The information on the relation of parathyroid function to metabolism is so meagre and unorganizable, that definitive analysis of the mechanism of the growth response to its deficiency, such as was given in the case of the thyroid, must be restricted. While it is true that the removal of the parathyroid glands results in a toxemia, and that it is this toxemia which is at the basis of the growth retardation, the origin, nature and ramifications of influence of the toxic products is still unknown.

The fact of tetany shows that a large part of the influence of the toxemia is exerted through the nervous system. While the cause of the tetany is as yet undetermined, although the recent work of Collip (25) and Hjort, Robison and Tendick (26) indicate that calcium deficiency is a major factor, it is clear that the condition, whatever its cause, is potentially productive of an enhanced utilization of metabolites which might otherwise be used in part in growth. This is, then, a condition which might well contribute to a nutritional inadequacy, and which would not be negligible even though the tetany be mild and chronic.

Now just as the toxemia may be presumed to exert a disturbing influence upon the skeletal neuro-muscular system and utilization of metabolites, so is it probable that it has a disturbing influence upon the visceral neuro-muscular system and the organs concerned in the digestion, absorption and preparation of foodstuffs for assimilation and utilization.

Carlson (27), (28) and Carlson and Jacobson (29) observed a depressive effect of parathyroid deficiency on the motility of the gastro-intestinal tract, anorexia, diarrhea and retardation or inhibition of digestion: Friedman (30) and Spadolini (31) found degenerative changes in these organs under similar conditions: Keeton (32) reported a decrease in gastric secretion accompanied by a diminution in its digestive power during tetania parathyreopriva, and that an improvement occurs in both factors during the depression which follows the tetany: Stoland (33) observed a decrease in bile and pancreatic excretion, while Artom (34) reported a progressive diminution in the amount of intestinal secretion as well as of its invertase and erepsin content.

The picture yields the basic assumption that the retardation of growth in conditions of parathyroid deficiency is a consequence of an inadequate nutritional level within the organism.

As stated earlier, expression of the growth response to parathyroid deficiency, as set forth in the curves of the percentage rate of growth, is, in general, quite like that exhibited when thyroid is lacking. There is the same consistent tendency to adjustment to the values characteristic of the age or stage of development after the initial period of distortion, and there is the same appearance of a percentage rate of growth less than that characteristic of the body weight.

Figure 4 is given to illustrate this similarity. It shows the percentage rate of growth on age of the male rats parathyroidectomized at 23 days of age and their controls: and the same data for the males operated at 100 days. Furthermore the absolute rate of growth is less than that to be expected for the age and weight.

No detailed explanation is necessary as to how a lowering of the nutritional level acts to produce a decrease in the absolute rate of growth below the level characteristic of the age and weight, nor why the percentage rate of growth is less than that to be expected for the body weight. The former is self evident, and the mechanism of the development of the latter has already been given.

From the fact that the percentage rate of growth tends to become characteristic of the stage of development after a short period of distortion, it is evident that the toxemia of parathyroid deficiency and the resultant lowering of the nutrient level within the organism, does not alter the effectiveness of the factors concerned in the regulation of the intensity factor of growth, and that the parathyroid hormones are not directly concerned therewith.

The fact of adjustment might be taken as indicating the presence of accessory parathyroid tissue functionally capable of taking over the work ordinarily carried on by the thyroid-associated glands. This objection has been met in an earlier paper (20). Since that time Hoskins and Chandler (35) have published the results of an exhaustive study of accessory parathyroid tissue in the albino rat. Their conclusion is—"accessory parathyroids are rare in the rat, and that this animal furnishes favorable material for the study of the results of parathyroidectomy."

The reëstablished equilibrium is however less stable in the case of parathyroid than in the case of thyroid deficiency. This is shown by the fact that during the second period, the deviation of the percentage rate of growth of the tests from the controls is greater in the parathyroidless than in the thyroidless groups in practically all the age series.

The instability is what might be expected if growth-inhibiting toxic substances accumulate to a certain level and then are disposed of in some way or other. This hypothesis was developed in an earlier paper (1). It is supported by the individual growth records of the study as a whole, by the common observation that parathyroidless animals suffer attacks of acute tetany between which there are periods of quiescence, and by the fact that teeth defects have occurred,—been repaired,—occurred again,—been repaired and occurred yet again in one and the same parathyroidless rat during the growth period subsequent to the glandular extirpation.

No inference is to be drawn from this instability that the reattainment of the percentage rate of growth characteristic for the age is fictitious and not real. True it is that the hewing to the line of the normal is probably not as uniform as the charts would indicate because of arithmetical compensations occurring in the combination of all test records for a given age series into one group. Nevertheless the adjustment is real though subject to temporary dislocations for the reason given above. The curves of the individual percentage rates of growth are uniformly less than the expected on the basis of weight, and weave around those for the controls on the basis of age after the preliminary period of adjustment.

Turning to the first period, or the period of adjustment, it is found that the initial reduction response to parathyroid deficiency is greater than that which follows thyroid removal. This is due to the partial inanition caused by the non-lethal acute tetany which develops immediately after parathyroid removal: a condition which is not obvious in the thyro-parathyroidectomized animals for reason given earlier; and which soon passes over, after a period of depression, into a mild, latent tetany. The latter is detectable by the fine tremulousness of the animal when handled. This phenomenon is not noticeable in the control or thyro-parathyroidectomized rats. Acute tetany attacks occasionally occur during the succeeding period of observation. The severity of the immediate cut in the per-

centage rate of growth may possibly be enhanced by an exacerbation of thyroid influence following the traumatic irritation to the gland due to parathyroid removal.

The percentage rate of growth is immediately accelerated after the initial reduction, as shown by the rising curve on chart 4. The reaction is common to all age groups. It is indicative of an increase in the nutrient level brought about by an improvement in digestive activities (32). It is quite consistent with Robertson's (6) hypothesis in relation to the growth response on readmission of food after a period of inanition, and is the explanation of the cyclical character of the percentage rate of growth exhibited by the individual parathyroidectomized rats (1).

The new nutritional level attained after the primary inhibition is, however, less than the normal because of a persistence of the toxemia though in milder degree.

This means that lesser amounts of materials are worked over by the organism in a given period. This then is essentially the same situation as occurs in conditions of thyroid deficiency. It can therefore be assumed that the mechanism of the adjustment is practically the same in the case of parathyroid as in the case of thyroid deficiency. The facts show that in both cases the capacity factor for growth (absolute rate of growth: amount of materials worked over in a given time) is reduced, and that in both cases the intensity factor (the percentage rate of growth: the amount of increment per unit initial mass per unit time) maintains the value characteristic of the stage of development, after a short period of adjustment. Since the basic change in both cases has been one in capacity rather than in intensity the interpretation of the mechanism of adjustment from primary dislocation of relations should be the same in one case as in the other. The cause of the change in capacity differs of course in the two cases, as has been outlined above. A difference in degree of response is, however, indicated. With certain exceptions to be noted later the degree of total retardation for the period of observation is less in cases of parathyroid than in cases of thyroid deficiency. Moreover the duration of the period of adjustment is less in the former than in the latter. The difference in favor of the parathyroidless animals is an expression of a lesser lowering of the capacity factor. It can be attributed to a less generalized influence of the disturbing process and to the occasional partial release of the digestive activities from the inhibiting toxemia.

To my mind the most significant thing brought out by the study as a whole from the biological point of view is the beautiful power of the living organism to adjust itself to these adverse conditions and maintain its percentage rate of growth regardless of the diminution in the amounts of material worked over in a given time.

I have purposefully omitted discussion of the possible accumulation of

toxic products in the parathyroidless animals as noted in an earlier paper (2) because this reaction is apparently only a factor during the late pubertal stage of development. The matter will be taken up in a succeeding paper.

As in the thyroidless so in the parathyroidless groups the onset of puberty brings a change in the degree of response to glandular deficiency. There is the same abrupt drop in the ratio of test to control growth as determined from the averages of the weekly percentage rates of growth for the period of adjustment: as in the increase (after this time) in the difference between tests and controls with increasing age of the animal at the time of initiation of the glandular deficiency.

This confirms the hypothesis that the mechanism of growth retardation is, in both types of glandular deficiencies, due to the same underlying course, viz., a reduction in the amount of materials worked over, and hence supports the conclusion that thyroid influence in growth is practically entirely a matter of its participation in the regulation of the metabolic level.

Now the similarity in the changes at puberty show that the change in growth response to thyroid deficiency at this time is due more largely to a change in need for growth materials directly brought about by pubertal influences on metabolism, than to the loss of an enhanced thyroid participation in growth over the normal progressive increase which occurs with age.

At puberty a new stimulus to growth occurs: in the intact animal this stimulus is added to those already present: because of it an increased call for building materials is made by the organism: these are not only not available in the animals operated during the period of production of the added stimulus but the supply is absolutely decreased: the expression of the new stimulus is inhibited: hence growth as compared to normal is doubly retarded. First because of the decrease in the amount of materials worked over during a given period, and second because of the inhibition to the expression of the new growth impulse.

The possibility must be recognized that the loss of a possibly enhanced thyroid activity, due to gonadal incretory exacerbation, which would tend to raise the metabolic level and hence the amount of materials worked over is contributive to the reaction in the thyroidless groups; and that in the parathyroidless groups a contributive factor might be an increased sensitivity of the vegetative nervous system to the toxic products which would result in an added decrease in available materials because of an enhanced disturbance of the processes of digestion, preparation and absorption of foodstuffs.

It is probable that the pubertal growth stimulus, presumably gonadal in origin, is a factor in the adjustment discussed in earlier paragraphs of the percentage rate of growth to the value characteristic of the stage of de-

velopment. Evidence for the belief is had from the fact that puberty, as already shown, brings about an equilibration and stabilization of the percentage rate of growth in body weight and length, and in bone weight, length and chemical differentiation in the normal animal: and from the fact that the point of attainment of adjustment in the rats operated at 23 to 30 days of age (before puberty) is in general about the time when the normal animal is well into adolescence (figs. 3 and 4). The attainment of equilibrium is not coincident with the beginning of puberty because the decrease in materials available for growth such as occurs in cases of thyroid deficiency (36) and in cases of restricted diet (37) retards the physiological development of the reproductive system. The acceleration of the percentage rate of growth noted earlier in the thyroidless rats of the 50, 65, 75 and 100 day old series may well be factored by a growth stimulus emanating from gonadal activity according to the interpretation given in an earlier portion of this paper.

Although gonadal incretory activity is undoubtedly concerned in the growth phenomena of puberty, I distinctly do not wish to give the impression that I believe this is the direct activating agent of the acceleration. How or through what path the influence is exerted is unknown and this is no place to indulge in speculation on the matter. The observations here recorded do show that the path is largely other than through the thyroid and that is all that can properly be written at this time.

It is well to call attention to the fact that the data on which this study is based are derived from two separate sets of records: one of male and one of female rats. The growth reactions subsequent to glandular removal at the different ages are common to both sexes. This consistency in the two sets of observations makes them mutually confirmatory and establishes the reliability of the data.

The purpose of this paper has been to give the picture as a whole and the generalizations justified therefrom. The avoidance of details has been purposeful because their multiplicity would make any recital contributive to confusion rather than to clarity. It is to be remembered that the study is of the rôle of the thyroid in *growth*, and not of its part in metabolism. Because of this I have omitted any correlation of the data or the conclusions derived therefrom with the latter process, preferring to stick to the original design. The interpretations are consistent with the findings in this other field of investigation.

CONCLUSIONS

Six basic principles evolve from these observations:

1. The rôle of the thyroid in growth is that of a participant in the regulation of the metabolic level through which is determined the rate of the maintenance processes of the organism and hence the amount of material

presented to the cells for growth purposes during a given period. It is therefore a determinant of the capacity factor in growth.

2. Catalysts other than those produced by the thyroid are concerned in the determination of the intensity factor in growth.

3. The thyroid is more largely concerned in growth by increase in cell mass than in growth by increase in cell number.

4. The parathyroids are not concerned in growth except in so far as they protect the organism from the growth retarding influences exerted by toxic products resulting from parathyroid deficiency. The retardation of growth which obtains is largely attributable to a lowering of the nutrient level following a disturbance in effectiveness of the digestive system due to the response of the sympathetic system to the toxemia.

5. Puberty has in influence upon the growth response to the glandular deficiencies. This influence originates in gonadal incretory development and is expressed as an increase in the call for growth materials.

6. The fact of most significant biological interest is the demonstration of the stability of the intensity factor of growth under adverse conditions and the remarkable power of the organism to adjust itself in this respect.

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GASEOUS EXCHANGE BETWEEN THE BLOOD AND THE LUMEN OF THE STOMACH AND INTESTINES

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The object of the physiologist, and ultimately of the clinician, is to reduce to their simplest terms the observed phenomena of the body. Owing to the complexity of the human system, the ideal is rarely attained. Perhaps the nearest approach to such a goal is reached in regard to the behavior of gases in the organism. Most of the work on this subject has centered around the process of respiration; although the behavior of gases in the coelomic and pleural cavities and subcutaneous tissues have been investigated,—more recently by Campbell (1925a), Haggard and Henderson (1919), Teschendorf (1924) and others. The behavior of gases in the gastro-intestinal tract has also been the subject of numerous investigations; and an extensive bibliography of the work in this field has been collected by Kantor (1918). It is generally admitted that the atmospheric air entering the stomach during swallowing and the gases arising in the intestinal tract by fermentation are modified by interchange with the gases of the blood stream. The present work was undertaken to supply more exact data upon the factors governing this exchange.

In order to demonstrate the fundamental points of the movement of gases in general, experiments upon the behavior of CO_2 in the stomach are presented in detail in the first part of this paper. Carbon dioxide was selected for our basic study for the following reasons. In the first place, its diffusion constant in the tissues has already been determined. Secondly, it diffuses sufficiently rapidly to permit the establishment of virtual equilibrium in the course of an acute experiment. This is of particular importance if the data are to be interpreted on a mathematical basis. Thirdly, the respiratory center may be expected to stabilize the tension of CO_2 in the arterial blood. Fourthly, the buffering system of the blood minimizes the change in tension of CO_2 as the blood traverses the capillaries.

The exact mechanism of the entrance of CO_2 into the lumen of the gastro-intestinal tract has been a moot point. The controversy as to whether an active secretion of CO_2 into the stomach occurs had apparently been settled in the negative by Planer (1860); but the question was reopened by Schier-

beck's work (1892) on the high tension of CO_2 found in the stomach of the dog during digestion. Edkins (1922) repeated Schierbeck's experiments with a modification in technique,—obtaining gastric activity by the injection of gastrin. The conclusion from this work was that it was unnecessary to assume any secretory activity, passive diffusion being sufficient to explain the presence of CO_2 in the stomach. In a later paper, Edkins and Murray (1924) advanced the idea that under certain conditions a "one-way" diffusion may occur. Gaseous exchange in the human subject has been studied by Ylppö (1916), Dunn and Thompson (1923) and others.

THE THEORY OF GASEOUS DIFFUSION. If the exchange of gas between the lumen of the gastro-intestinal tract and the blood circulating in its wall is due to the physical process of diffusion, it must conform to the following criteria. First: an equilibrium will be reached when the partial pressure of gas in the lumen is equal to the mean tension of the gas dissolved in the circulating blood. This condition of equilibrium should be the same, regardless of the direction from which it is approached. Second: the exchange of gas which leads to the development of this equilibrium will follow a course determined by the law that the rate at which gas passes through a permeable membrane at any time will be proportional to the difference in pressure of this gas on the two sides of the membrane. Third: the actual value of the rate at which the gas passes across the membrane is, for any given pressure difference, determined strictly by the properties of the gas and by the area and thickness of the membrane.

The general theory from which these criteria arise is based on the experimental work of Exner (1875), Stefan (1878) and Hüfner (1897), amplified in its application to the oxygenation of blood in the lung by Bohr (1909) and A. and M. Krogh (1910 and 1915). The quantity of a given gas which will diffuse across a thin membrane in unit time depends directly upon the difference in partial pressure of the gas on either side of the membrane, the extent of its surface, and inversely upon its thickness. The absolute value of the rate of diffusion when these conditions are defined is determined by the characteristics of the gas in question and by the nature of the membrane through which it is diffusing. In applying these facts to the diffusion of CO_2 across the gastric mucosa, we may proceed as follows.

Let a equal the mean tension of CO_2 in the blood in the capillaries of the mucosa measured as a per cent of one atmosphere;
Let x equal the partial pressure of CO_2 in the viscus similarly measured;
Let S equal the surface of the viscus measured in cm.^{2};
Let V equal the volume measured in cm.^{3};
Let l equal the effective thickness of its wall measured in μ; and
Let D equal the diffusion coefficient of CO_2—defined as the volume of this gas which will diffuse through 1 cm.^{2} under a pressure gradient of 1 atmosphere per 1μ thickness in 1 minute.

The quantity of CO_2 in the viscus is given by $x \cdot V$ and the rate of diffusion by

$$\frac{d(x \cdot V)}{dt} = \frac{D}{l} \cdot S \cdot (a - x) \tag{1}$$

If we may assume that in the course of an experiment a, V, S, and l do not change significantly, we may write

$$\frac{dx}{dt} = \frac{D \cdot S}{l \cdot V} (a - x) \tag{2}$$

and letting

$$k = \frac{D \cdot S}{l \cdot V} \text{ and substituting} \tag{3}$$

$$\frac{dx}{dt} = k(a - x) \qquad \text{and} \tag{4}$$

$$k = \frac{1}{t} \log_e \frac{a}{a - x} \tag{5}$$

In this form the equation can be applied to experimental data, provided the experiments are so executed that the foregoing assumptions are valid, —i.e., that the diffusion of CO_2 into or out of the viscus does not take place in sufficient quantity to modify its dimensions very much.

THE EXPERIMENTAL METHOD. The application of the foregoing theory to the movement of CO_2 across the gastric wall has been tested by two types of experiment. 1, Air, either free from CO_2 or rich in CO_2, has been introduced into the stomach, and the changes in its concentration noted as it approached equilibrium with the CO_2 in the blood. 2, The partial pressure of CO_2 in the blood has been varied abnormally and observations made on the changes in concentration of CO_2 in the gaseous content of the stomach as a new equilibrium was reached. All of the experiments were carried out upon the anesthetized cat. About 50 mgm. of amytal per kilo of body weight were administered by intraperitoneal injection. The animal was given no food for from twenty-four to forty-eight hours prior to the experiment, but had free access to water. After anesthetization, the abdomen was opened, the stomach gently washed out with warm water, and the pylorus and cardia ligated, care being taken not to injure the blood supply. The body temperature was recorded by a rectal thermometer and a constant level maintained with a heating pad. The gas to be studied was then injected, to the amount of 60 cc. After the lapse of varying intervals of time, samples were drawn off in well-oiled Luer syringes provided with needles and short rubber connections, and were analyzed upon a portable Haldane gas analyzer. It was customary after drawing off two

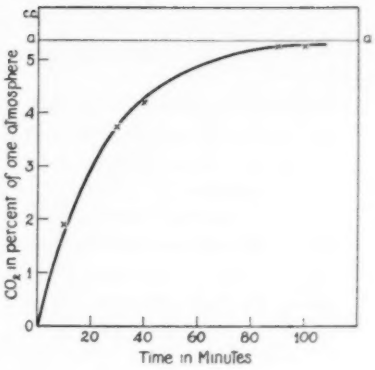


Fig. 1. Observed course of diffusion of CO_2 into the stomach, compared with the theoretical curve obtained from equation (5) (see text). a, the mean partial pressure of CO_2 in the blood, is assumed to be 5.38 per cent of one atmosphere (line a---------a). k has the value of 0.0394. The crosses indicate the percentages of CO_2 in the stomach, determined at various periods of time following the introduction of 60 cc. of air into the stomach.

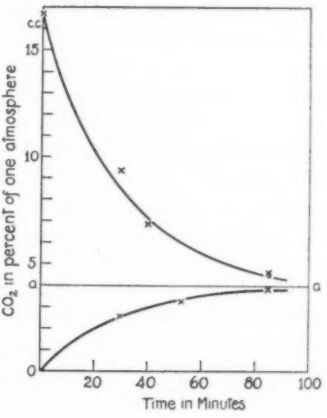


Fig. 2. Comparison of course of diffusion into and out of the stomach, showing in each case that observed measurements are in close accord with the theoretical curve calculated from equation (5). This implies that the velocity of diffusion is at all times in proportion to the head of pressure. The tension of CO_2 in the blood (line a---------a) is assumed to be 4.0 per cent of one atmosphere; the value of k is 0.0349 for the lower curve, 0.0344 for the upper. The crosses related to the ascending curve indicate the percentages of CO_2 found in the stomach following the introduction of air, as in figure 1. After this curve was obtained, the gas was withdrawn from the stomach and replaced by a mixture of air containing 16.7 per cent of CO_2. CO_2 now diffused from the lumen of the stomach into the blood, as shown by the crosses along the descending curve.

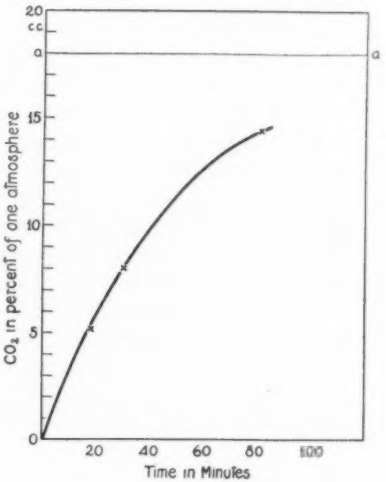


Fig. 3. Course of diffusion of CO_2 into the stomach when the percentage of CO_2 in the blood is increased. Again, the curve is calculated from equation (5). The CO_2 in the capillary blood is assumed to be 18 per cent of one atmosphere (see text).

samples of about 10 cc. each to empty the stomach and refill it with a fresh 60 cc. of the gas, so that additional samples could be withdrawn without greatly decreasing the volume of gas in the viscus.

Measurements of the surface area and the thickness of the mucosa of the cat's stomach under the conditions of the experiments were made in order that the rate at which the gas passes across could be calculated in absolute dimensions. This was done as follows. The cardia and pylorus having been ligated, the stomach was injected, *in situ*, with 60 cc. of formol-alcohol, which has been shown by Patten (1920–21) to result in a minimum of shrinkage. It was then removed and fixed for twenty-four hours in the same solution. The surface area was computed from a series of direct measurements and this was checked by comparing the weight of a square centimeter of the stomach wall with the total weight. Blocks were cut from the region of the fundus, cardia and pylorus, and sections prepared both by freezing and by paraffin-embedding. Measurements of the thickness of the mucous membrane were made for the three areas of the stomach referred to above. The camera-lucida drawing (fig. 4) is of a section from the fundus. The specimen for figure 5, showing the arrangement of the capillary circulation in the mucosa, was prepared by placing a cannula in the gastric artery of the anesthetized cat and injecting Berlin blue at a pressure of 200 mm. Hg.[1]

Results. Figure 1 illustrates the result of an experiment in which 60 cc. of air were introduced into the stomach of a cat. The crosses indicate the per cent of CO_2 in the stomach, determined at various periods of time following its introduction. The curve is calculated from equation (5), assuming a, the mean partial pressure of CO_2 in the blood, to be 5.38 per cent of one atmosphere. k has the value 0.0394. This experiment is selected as showing especially well the agreement between the experimental points and the theoretical values which may be calculated for them. It is clear that the partial pressure of CO_2 in the stomach is approaching, and after 90 minutes has nearly reached, a limiting value which cannot differ greatly from the pressure of CO_2 in the capillary blood.

Figure 2 illustrates the result of an experiment in which the equilibrium concentration was approached from above as well as below in the same experimental animal. Sixty cubic centimeters of air were first introduced into the stomach and allowed to come into equilibrium with the blood as shown by points along the lower curve. After 85 minutes the gas was withdrawn completely from the stomach and replaced by a mixture of air containing 16.7 per cent of CO_2. Carbon dioxide now diffused from the lumen of the stomach into the blood as shown by the points along the upper curve. Both curves are constructed from equation (5), assuming

[1] We are indebted to Dr. H. S. Forbes for his kindness in making this injection for us.

the tension of CO_2 in the blood to be 4.0 per cent of one atmosphere. The experiment shows that the limit is the same no matter in which direction CO_2 is moving as equilibrium is approached. The value of k is 0.0349 for the lower curve; 0.0344 for the upper. Carbon dioxide, consequently, diffuses in either direction with equal velocity under comparable heads of pressure. The experiment of August 5 in table 1 is in confirmation of this conclusion.

In the experiment illustrated in figure 3, the conditions for diffusion have been modified by causing the cat to breathe air containing about 16 per cent of CO_2. Air was then introduced into the stomach and its composition determined after 18, 30 and 81 minutes. A curve corresponding to equation (5) may be drawn through the points so determined, if a, the tension of CO_2 in the capillary blood, is taken to be 18 per cent of one

TABLE 1

DATE	INSPIRED GAS	CO_2 PER CENT IN INJECTED GAS	CO_2 PER CENT APPROACHED AS LIMIT	
		x_0	a	k
May 2	Air	0	5.34	0.0602
May 21	Air	0	5.38	0.0394
July 31	Air + 16 per cent CO_2	0	18.00	0.0196
August 1	Air	0	4.00	0.0349
		16.7	4.00	0.0344
August 5	Air	0	4.5	0.0393
		12.35	4.35	0.0410
Mean				0.0384

atmosphere. This value is two per cent higher than that of the inspired air and does not differ much from what one might expect.

These experiments should serve to demonstrate satisfactorily that the exchange of CO_2 across the wall of the stomach conforms to the first two criteria which we laid down for the process of diffusion. We must now examine whether the actual value of the rate at which CO_2 passes across the membrane is determined strictly by the diffusion coefficient of this gas and the area and thickness of the lining of the stomach. The problem reduces itself to the question whether k, the diffusion coefficient for the stomach as a whole, which we have seen to be subject to experimental determination, can be predicted in terms of the diffusion coefficient of CO_2 in tissue and the anatomical dimensions of the stomach. Table 1 contains the collected data from the three experiments described above, together with two others, in regard to the value of k.

The mean value of k is 0.0384. Experiments on three animals yield

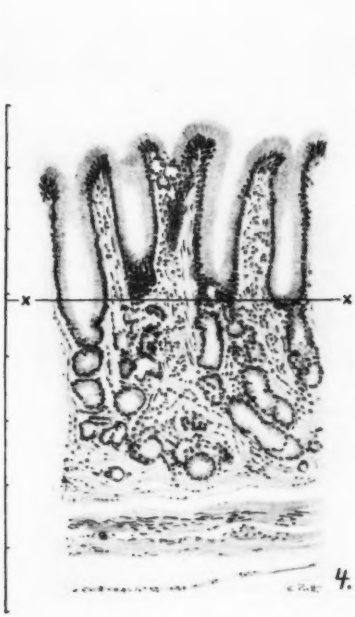
results close to this average; two others differ by not more than 50 per cent from it.

Equation (3) states that $k = \frac{D \cdot S}{l \cdot V}$. The diffusion constant of CO_2, D, has been estimated from the experiments of Krogh (1919), who has determined the diffusion constant for O_2 in animal tissues to be 0.133 at 20°C. Krogh states that this value increases 1 per cent for each degree of rise in temperature. At 38°C. the diffusion coefficient of O_2 would consequently be $0.133 \times 1.18 = 0.136$. The relative rates of diffusion of O_2 and CO_2 are found by Krogh to be 1:35.7. The diffusion coefficient of CO_2 may consequently be taken as $0.136 \times 35.7 = 4.86$.

The method of determining the surface of the stomach has been described. The value obtained was 125 cm.^{2} and is probably accurate to 15 or 20 per cent. The volume of the stomach was fixed at the beginning of each experiment at 60 cc. by the injection of that quantity of air. This volume probably was not modified by more than 5 per cent from the exchange of gas with the blood but was diminished somewhat by the taking of samples for analysis.

The value of l, the thickness of the membrane through which the exchange takes place, is not so readily measured. In the first place, the lining of the stomach is not a smooth, continuous sheet of epithelium, but is of a shape infinitely complicated by the gastric glands as well as by grosser irregularities of the mucosa as a whole (fig. 4). Furthermore, the epithelium is not underlaid by a continuous sheet of blood, as the theoretical treatment presupposes, but by a rather attenuated system of capillary loops extending into the spaces between the gastric pits (fig. 5). We have been forced, consequently, to abandon the attempt to estimate k from the dimensions of the stomach and have resorted to determining l from the other factors in equation (3). We may call l the virtual thickness of the membrane through which diffusion occurs and test our proposition by seeing whether it has a magnitude which is reasonable. Clearly l must be greater than the thickness of the epithelium which covers the mucosa (40μ) and probably should be considerably less than the total thickness of the mucosa (500–600μ). Substituting the values obtained for the other factors in equation (3), we find $0.0384 = \frac{4.86 \times 125}{l \times 60}$ or $l = 264\mu$. This brings the virtual thickness of the membrane well within the limits which we have selected as reasonable. The inner surface of this virtual membrane lies at the depth of the bottom of the gastric pits, as the line $x - - x$ drawn across figure 4 indicates. This most rigorous test of the diffusion hypothesis is met as closely as the inherent difficulties of the situation allow. The theory of diffusion is adequate to account for every detail of our experiments on the movement of carbon dioxide into and out of the cavity of the stomach.

PART II. Having established the factors noted in part I in regard to CO_2, we proceeded to study the behavior of some of the other well-known gases encountered in the intestinal tract. This subject is of interest not only in its theoretical aspect but in its practical bearing upon postoperative distention and other forms of intestinal meteorism.



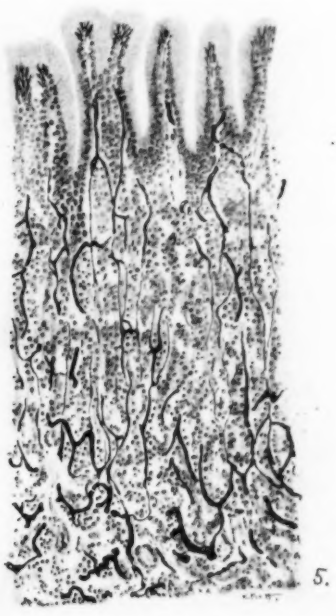


Fig. 4. Camera-lucida drawing of a section of gastric mucosa; magnification about 200 diameters. The divisions on the scale to the left are equivalent to 100μ. The line x---------x is laid off at a depth of 264 μ, which is the value of l derived from equation (3) (see text). The line passes through at the depth of the gastric pits and shows that the calculated value for the thickness of the membrane (l) through which diffusion is taking place is reasonable when compared with the anatomical structure of the gastric mucosa.

Fig. 5. Camera-lucida drawing showing the arrangement of the capillary circulation in the gastric mucosa; magnification about 200 diameters (see text).

THE METHOD. The same conditions as regards handling the gases and maintaining the body temperature of the animal were observed as those described in part I. The Haldane apparatus for gas analysis was equipped with a combustion chamber. The anesthetic used in most cases was urethane, from 4 to 6 cc. of a 25 per cent solution per kilo of body weight

TABLE 2

DATE	WEIGHT OF CAT	ANESTHETIC (ETHER, URETHANE, AMYTAL)	VISCUS	GAS INJECTED	VOLUME OF GAS INJECTED	TIME INTERVAL	VOLUME OF GAS WITHDRAWN	ANALYSIS OF GAS WITHDRAWN					AMOUNT OF INJECTED GAS ABSORBED	REMARKS
								CO_2	O_2	N_2	H_2	CH_4		
	kgm.				*cc.*		*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	
1-16-24	2.5	U	25 cm. loop small intestine	O_2	20	1 hour	8.5	5.0	73.5	21.5	0	0	13.7	Loop ligated with tape, taking care not to injure blood supply
3-13-24	3.2	U	28 cm. loop small intestine	O_2	20	2 hours	6.0	5.1	63.5	31.4	0	0	16.2	Absorption slower in 2nd hour because pressure and volume have decreased
1-16-24	2.5	U	Stomach	O_2	50	1 hour	49.0	6.8	88.3	4.9	0	0	6.7	Ligatures at cardia and pylorus
3-13-24	3.2	U	Stomach	O_2	50	3 hours	37.0	6.6	82.7	10.7	0	0	19.4	Ligatures at cardia and pylorus
1-17-24	2.5	U	13.5 cm. loop colon	O_2	17	1 hour	14.0	6.6	78.6	14.8	0	0	6.0	Length of loop about half that of small intestine, Jan. 16
6-29-25	2.5	A	25 cm. loop small intestine	CO_2	20	3 minutes	12.0						8.0	The 3 experiments with CO_2 show the rapid absorption of this gas from different portions of the gastro-intestinal tract
1- 4-24	2.7	U	Stomach	CO_2	50	15 minutes	11.0						39	
1-18-24		U	20 cm. loop colon	CO_2	30	10 minutes	6.5						23.5	
6-29-25	2.5	A	21.5 cm. loop small intestine	N_2	20	1 hour	20.3	5.2	2.0	92.8			± 1	Contrast absorption rate of N_2 with that shown in table 4
6-29-25	3.5	A	26 cm. loop small intestine	N_2	20	2 hours	20.5	5.3	2.9	91.8			± 1	
3-10-25	2.3	U	30 cm. loop small intestine	N_2	20	6 hours	14.0	4.9	5.7	89.4			7.5	

1-17-24	2.5	U	Stomach	N_2	50	1 hour	52.5	4.2	1.4	94.4			± 1	
2-23-24	3.0	U	25 cm. loop small intestine	H_2	20	1 hour	21.0	1.6	2.9	34.5	61.0		7.2	Respiration very rapid throughout experiment
2-28-24	2.5	U	33 cm. loop small intestne	H_2	22	2 hours	24.0	4.4	9.0	36.4	50.2		10.0	
2-28-24	3.3	E	30 cm. loop small intestine	H_2	20	7.5 hours	15.0	6.6	15.0	32.1	46.3		13.1	Animal etherized, loop of intestine isolated and H_2 injected, abdomen closed. At end of 7½ hours animal again etherized and abdomen opened. Gas withdrawn
4-30-24	3.0	A	49 cm. loop small intestine	CH_4	25	1 hour	24.0	3.0	5.0	5.7	0	86.3	4.3	Loop washed out with warm salt solution before injection of gas
4-30-24	3.0	A	30 cm. loop small intestine	CH_4	25	3 hours	18.0	4.7	7.4	18.4		69.5	12.5	
3-10-24	2.3	U	30 cm. loop small intestine	H_2S	10	2.6 minutes	7.0						3.0	See figure 7
6-29-25	2.3	A	32 cm. loop small intestine	Air	20	1 hour	18.2	5.2	9.3	85.5				Contrast volume changes with table 5
6-29-25	3.5	A	32 cm. loop small intestine	Air	20	2 hours	17.0	4.9	4.5	90.6				Note small amount of absorption
3-14-24	3.1	U	45 cm. loop small intestine	Air	25	5 hours	21.0	2.9	14.2	82.9				
5- 2-24	2.9	A	Stomach	Air	60	1 hour		5.2	15.3	79.5				Volume not estimated

being injected intravenously; in a few instances amytal was used, injected intraperitoneally in doses of about 50 mg. per kilo of body weight.

The gases studied were O_2, N_2, H_2, CH_4, H_2S and atmospheric air, with further studies upon CO_2: a list embracing gases which are natural constituents of air and combustible gases arising from fermentation and putrefaction. The rates of absorption of these gases and the equilibria established between them and the blood gases were studied by introducing each gas separately into an isolated intestinal loop or into the stomach. The major work was carried out upon the intestines, because a number of the gases studied are found there commonly and are encountered in the stomach only under pathological conditions. Our preliminary experiments showed no great difference in the absorption rate between the colon and the small intestine, and it was found more convenient to work with the latter.

TABLE 3

GAS	EXNER FIGURES $\frac{\alpha^{40°C.}}{\sqrt{d^{40°C.}}}$	OBSERVED ABSORPTION TIME OF 100 CC. FROM PERITONEAL CAVITY OF THE DOG	OBSERVED ABSORPTION TIME OF 600 CC. FROM PLEURAL CAVITY OF THE DOG	OBSERVED ABSORPTION FROM INTESTINAL LOOP OF THE CAT
N_2	0.018048	3–4 days	20–26 hours	±1 cc. (1 hour)
O_2	0.02195	20–24 hours	10–12 hours	13.7 cc. (1 hour)
CH_4	0.0948			4.3 cc. (1 hour)
H_2	0.063853	22–24 hours	7–10 hours	7.2 cc. (1 hour)
CO	0.018048	16–18 hours	6–8 hours	
C_2H_6	0.0284	7–9 hours	2–3 hours	
N_2O	0.4414	1–1½ hours	½–1 hour	
CO_2	0.4293	45–90 minutes		8 cc. (3 minutes)
C_2H_2	0.8809	25–30 minutes		
H_2S	1.513	3–5 minutes		3 cc. (2.6 minutes)

Columns 3 and 4 show Teschendorf's observed absorption times; the last column shows our own.

The procedure was as follows. A portion of intestine about 25 cm. long was selected, and in certain cases was gently washed through with warm water; in other cases, when it was perfectly empty, this procedure was omitted. The loop was then ligated, great care being taken to avoid any interference with the circulation. Twenty cubic centimeters of the gas under consideration were injected, which caused a moderate distension of this length of intestine and were found to produce a pressure of about 15 mm. of mercury. The loop was then returned to the abdominal cavity and the wound closed with clips. After the lapse of certain definite intervals of time the loop was withdrawn and emptied of the gas, which was measured and analyzed. When the stomach was used, the pylorus and cardia were ligated as described in part I.

RESULTS. Typical experiments with each gas studied have been selected

and the results are given in the form of abbreviated protocols in table 2. Upon consulting the data thus presented it will be observed that when a gas is introduced into the gastro-intestinal tract and is then withdrawn after a lapse of time, it has undergone two important changes. First, a change in volume has taken place; secondly, a change in composition. These changes depend upon two fundamental processes: namely, the absorption of the injected gases, and a counterprocess of diffusion of the blood gases into the distended lumen,—the latter process continuing until an equilibrium is reached. The data obtained on the rate of absorption of the several gases will be presented first.

Rate of absorption. If we leave out of consideration for the moment the character of the membrane through which absorption is taking place,—that is, the cell membranes of the gastro-intestinal mucosa,—we may consider that the gas in the lumen of the intestine is surrounded by a layer of fluid not greatly differing from water when regarded as a solvent for the gases under consideration. The rate of diffusion of various gases through a surrounding layer of fluid has been shown by Exner (1875) to be proportional to the formula $\frac{a}{\sqrt{d}}$,—a being the absorption coefficient of the gas and d the density. Teschendorf (1924) in his work upon the absorption of gases from the peritoneal and pleural cavities compared his observed rates of absorption with the Exner numbers at a temperature of 40°C.; his results, taken from a table in his article, are shown in the first four columns of our table 3, while in the last column are given the absorption rates from the small intestine according to our present observations. It will be noted that an arrangement of the gases which takes into account the formula of Exner approximates that made on the basis of the observed rates of absorption. In considering the data of Teschendorf it must be remembered that determination of the gases diffusing from the blood into the lumen was not made; and while this counterdiffusion may be safely neglected in the case of gases that are absorbed with the rapidity of CO_2 and H_2S, where the whole process occupies only a few minutes, it is an important factor in the consideration of gases that are absorbed slowly. This will be considered in detail later.

The wide range shown in the rate of absorption of the several gases is striking; and in order to be able to compare these differences more readily, the relative rates of absorption from a 25-cm. loop are shown in graphic form in figure 6. In the case of CO_2, because of the rapidity of its disappearance, and of H_2S, because of its toxicity, the gas was withdrawn after the lapse of a few minutes, as stated in table 2, and the rate of absorption for one hour was calculated (for H_2S, see fig. 7). This is not strictly accurate, because the rate of absorption decreases as the volume diminishes; but, since the volume of the other gases is not greatly diminished at the end of one hour, the error introduced is probably not significant.

In considering the rate of absorption of gases, the character of the membrane across which diffusion is occurring must be taken into account. Teschendorf attributes great importance to this factor in explaining the differences which he found between the absorption from the pleural and that from the peritoneal cavity (see table 3). It is difficult to compare his findings in this respect with ours, since there is such a difference in surface area between the gastro-intestinal mucosa and the endothelial lining of the peritoneal and pleural cavities.

In the foregoing discussion of the absorption rate, the gases are considered to be chemically inert, and their relation to the blood controlled only by the physical laws governing the behavior of liquids and gases. This, of course, is not strictly the case with H_2S, CO_2 and O_2, which not only exist in physical solution in the plasma but also combine chemically

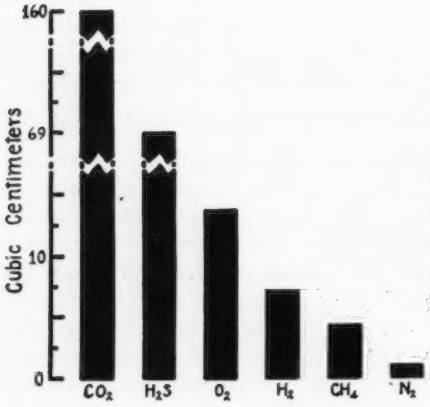


Fig. 6. Relative rates of absorption, expressed in cubic centimeters, of CO_2, H_2S, O_2, H_2 CH_4 and N_2 from a 25-cm. loop of small intestine. The height of the columns represents the number of cubic centimeters of the gas absorbed over a period of one hour. For method of estimating the rates of absorption of CO_2 and H_2S, see text.

with the constituents of the blood. For this reason these gases are absorbed more rapidly than is to be expected from the value of Exner's number. The problem is complicated by considerations involving the circulation rate through the mucosa and other factors and cannot be adequately handled with the available data.

It will be noted that N_2 has a very low rate of absorption. In explaining this, not only its low solubility in water must be considered, but also the fact that the blood and tissues are already saturated with N_2 at about four-fifths of an atmosphere of pressure.

In regard to H_2 and CH_4, gases foreign to the metabolism of the body, it will be noted that the intestine is capable of disposing of them in considerable quantities by way of the blood and lungs,—a fact emphasized by Tacke (1884), one of the early writers on this subject.

The toxic effects of H_2S are generally recognized. Figure 7 shows the

effect upon the systemic blood pressure of the injection of 10 cc. into an intestinal loop. The very rapid appearance of toxic symptoms is of interest, only about one and a half minutes elapsing between the injection of the gas and the fall in blood pressure. It should also be noted that the absorption rate of this gas is very rapid, its volume decreasing by about one-third in the course of a few minutes.

When atmospheric air is injected into the lumen of the intestine (table 2), the volume diminishes during the first and second hours, due to the absorption of O_2. As soon as the tension of O_2 in the lumen has approached that in the surrounding tissues, absorption proceeds slowly, only four cubic centimeters having disappeared at the end of five hours (table 2). Such slow absorption of atmospheric air is to be expected because of its close similarity in composition to the blood gases; and this is a fact of

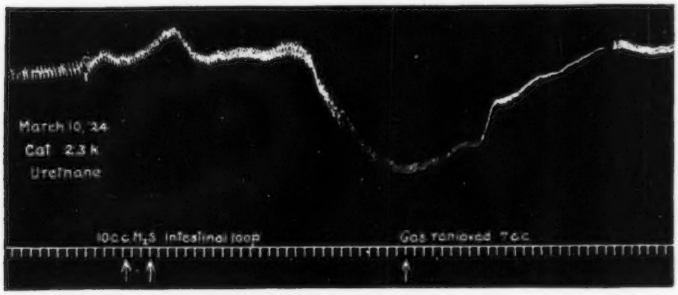


Fig. 7. Effect upon the systemic blood pressure produced by injecting 10 cc. of H_2S into an intestinal loop. The base line represents zero blood pressure; the intervals shown on the line are five-second periods. About one and a half minutes elapsed between the injection of the gas and the fall in blood pressure.

considerable clinical importance, since swallowed air taken into the stomach and passed thence into the intestines, is, we believe, a more important cause for certain types of distention than has previously been supposed. This belief has recently been confirmed by certain studies, to be published later, upon post-operative distention.

Change in composition of injected gas. When a loop of intestine is distended with a given gas a process is set up, the end point of which must be an equilibrium between the injected gas and the gases of the blood stream; so that in addition to the absorption of gas as already discussed there is also an entrance of gases from the blood into the lumen.

To illustrate the course of events following the injection of a gas into the lumen of the intestine, O_2, H_2 and N_2 have been selected as representing gases normally present in the body and those arising by fermentation. The changes following their injection are shown in detail in figure 8.

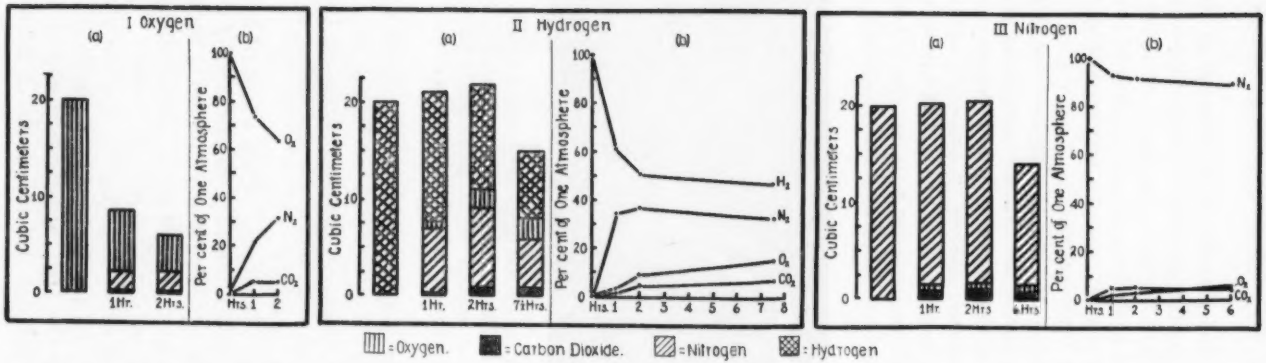


Fig. 8. Course of events following the introduction of (I) O_2, (II) H_2 and (III) N_2 into an isolated loop of small intestine. The gases were injected severally, allowed to remain for the period of time indicated, drawn off and analyzed. The first column in diagram *a* répresents in cubic centimeters the volume of gas injected; the succeeding columns represent the total volume of the gas and its composition when withdrawn from the intestinal loop after stated intervals of time. The percentage results of the analyses are plotted in diagram *b*. The line descending from the upper left indicates, in percentage, the absorption of the injected gas. The lines ascending from the lower left indicate the entrance of the blood gases.

Each diagram is composed of two sections. In sections *a* the results are shown in terms of cubic centimeters: the height of the columns represents the total volume; the divisions show the number of cubic centimeters of the several gases present. In sections *b* the points connected by lines represent the percentages of the different gases found when the samples were drawn off after the lapse of the intervals of time shown on the abscissae.

The first diagram of figure 8 shows the results of experiments with O_2. Twenty cubic centimeters of this gas were injected into the isolated intestinal loop. At the end of one hour, it will be noted, the volume had decreased from 20 cc. to 8.5 cc. On analysis, as shown in section *b*, the percentage composition was as follows: 73.5 per cent O_2, 5.0 per cent CO_2 and 21.5 per cent N_2. Carbon dioxide and N_2 had gained entrance from the blood, the CO_2 having practically reached equilibrium with the CO_2 of the blood. It will be observed that N_2 had entered in considerable quantities, due to the high tension at which it exists in the blood. At the end of two hours there was a slowing down of the rate of absorption of O_2, due to the diminished pressure difference and volume; the percentage of CO_2 showed little change; that of N_2 had increased still further, but not so rapidly as during the first hour.

The next diagram in figure 8 shows the results obtained with H_2. The importance of the diffusion of blood gases into the lumen is here clearly shown, for while at the end of the first hour 7.2 cc. H_2 had been absorbed, the total volume of gas in the intestinal lumen had slightly increased, owing to the entrance of CO_2, O_2 and N_2 from the blood; and at the end of the second hour a still further increase in volume is shown, although the amount of H_2 had continued to fall. After the lapse of seven and a half hours the percentage composition of the gas had not greatly changed; but absorption had considerably lowered the total volume.

On consulting the data shown in table 2, it will be seen that the behavior of CH_4 is in general similar to that described for H_2.

The last diagram shows the results with N_2. Reference has already been made to the high tension at which N_2 exists in the blood and in the air; therefore very little change is required to bring the N_2 injected into the lumen into equilibrium with that in the surrounding blood. It will be noted that the percentage had fallen to 92 at the end of one hour and remained close to this figure at the end of six hours. Oxygen and CO_2 entered from the blood stream, their percentage remaining relatively small.

The course of events when N_2 is injected into the loop and the animal connected with a spirometer containing O_2 is shown in table 4. Under these conditions one would expect that the tension of N_2 in the blood would fall and that a more rapid absorption of N_2 from the intestine would take place. If the rates given for N_2 absorption in table 2 be contrasted with

those shown in table 4, it will be observed that for the first hour the figures are the same, while for succeeding periods the absorption rates shown in table 4 are distinctly higher, as might be predicted. Another point of interest brought out by this experiment is the higher percentage of O_2 that gains entrance to the lumen of the intestine under these conditions, due to the increased amounts held in physical solution in the plasma and resulting in increased O_2 tension in the tissues. This confirms recent observations of Campbell (1925b) upon O_2 tension in the peritoneal cavity when the animal is breathing a mixture containing a high per cent of O_2.

TABLE 4

DATE	WEIGHT OF CAT	ANESTHETIC (ETHER, URETHANE, AMYTAL)	LENGTH OF INTESTINAL LOOP	GAS INJECTED	VOLUME OF GAS INJECTED	TIME INTERVAL	VOLUME OF GAS WITHDRAWN	ANALYSIS OF GAS WITHDRAWN			AMOUNT OF N_2 ABSORBED	REMARKS
								CO_2	O_2	N_2		
	kgm.		*cm.*		*cc.*	*hours*	*cc.*	*per cent*	*per cent*	*per cent*	*cc.*	
3-6-24	4	U	28	N_2	15	1	17	4.8	7.6	87.6	±1	Animal breathing from spirometer containing O_2; connection made by means of a T-shaped tracheal cannula fitted with Thiry-Tissot valves. Compare the absorption rate of N_2 from the intestine here with that shown in table 2 (see text)
3-6-24	4	U	38	N_2	22	2	22.5	7.1	8.4	84.5	3	
3-7-24	3.2	U	25	N_2	16	3	13	3.8	12.6	83.6	5.2	
3-7-24	3.2	U	30	N_2	20	6	11.5	5.7	8.7	85.6	10.2	

Effects of interference with the circulation. In addition to studying the behavior of the several gases in the intestines under normal conditions, certain experiments were carried out in which the circulation to the loop containing the gas was obstructed (see table 5). The circulatory disturbance was achieved either by ligating separately the mesenteric veins of the isolated loop, leaving the arterial supply undisturbed; or by the somewhat less drastic procedure of partially closing off the portal vein by means of a clamp provided with a set screw for fine adjustment. The clamp was closed until the intestine showed a faint cyanotic tinge. Following this procedure the gas to be tested was introduced.

The results are shown in table 5. The first two experiments, April 7,

TABLE 5

DATE	WEIGHT OF CAT	ANESTHETIC ETHER, URETHANE, AMYTAL	LENGTH OF INTESTINAL LOOP	GAS INJECTED	VOLUME OF GAS INJECTED	TIME INTERVAL	VOLUME OF GAS WITHDRAWN	ANALYSIS OF GAS WITHDRAWN				REMARKS	
								CO_2	O_2	N_2	H_2		
	kgm.		*cm.*		*cc.*		*cc.*	*per cent*	*per cent*	*per cent*	*per cent*		
4- 7-24	3.3	U	38	Air	30	1 hour	33	22	6.4	71.6		Increase in volume 3 cc. due to accumulation of CO_2	
4- 7-24	3.3	U	43	Air	25	2 hours	29	30	0	70.0		CO_2 high; all the O_2 has disappeared	Portal vein partially obstructed. Clamp adjusted so that intestine showed slight cyanotic color
5-21-24	2.5	A	25	H_2	25	1 hour	29	15	0	2.5	82	Increase in volume due to CO_2. H_2 absorbed, $\pm$ 1	
5-21-24	2.5	A	25	CO_2	25	10 minutes	11					Absorption rate of CO_2 about half that shown in table 2	
4- 4-24	3.4	U	52	Air	25	1 hour	32	26.3	4.8	68.9		Increase in volume 7 cc.	
4- 4-24	3.4	U	40	Air	25	2 hours	32	24.7	3.5	71.8		9 cc. of hemorrhagic exudate	
3-28-24	2.4	U	24	CO_2	20	15 minutes	11.5					Absorption rate about one quarter that in table 2	Mesenteric veins to the isolated loop ligated before injection of gas
3-28-24	2.4	U	27	O_2	20	1 hour	19	13.3	79.2	7.5		6cc. O_2 absorbed, intestine containing	
3-28-24	2.4	U	27	O_2	20	2 hours	15	30.1	57.8	12.1		O_2 pale in color; that containing N_2 deeply cyanotic	
3-28-24	2.4	U	43	N_2	30	4 hours	30	8.4	4	87.1			

1924, show the course of events following the injection of atmospheric air. Due to the interference with the circulation, the CO_2 content of the tissues rises rapidly and the diffusion of this gas into the lumen results in an increase in volume. It will be observed that this increase is partially offset by the disappearance of O_2, which is called upon to meet the increased demand for this gas by the tissues of the intestinal wall. In this connection the estimations by Boycott (1905) of the gaseous metabolism of the intestine of the rabbit are of interest. This writer considers the utilization of O_2 by the tissues of the intestinal wall a very important factor in the disappearance of this gas from the lumen. That this is true under the conditions of the experiments cited above (table 5) is evident, if the low percentage of O_2 found after one and two hours (April 4) be contrasted with the higher percentage found after the same intervals of time (table 2, June 29) when no O_2 deficiency of the tissues existed. This utilization of O_2 by tissues of the intestinal wall suggests the possibility of the introduction of this gas into the intestinal lumen or the peritoneal cavity in cases where serious O_2 deficiency exists; as for example after operation for intestinal obstruction complicated by interference with the blood supply of the intestine.

According to Boycott, CO_2 is formed in the intestinal wall of the rabbit at the rate of 3.7 cc. per cm. per hour. Under normal conditions this is a small amount, and negligible when we consider the rapidity with which CO_2 diffuses. The experiments in table 5 show, however, that it becomes significant when the circulation, which normally stabilizes the CO_2 tension in the tissues, is interfered with.

It will be noted (table 5) that the absorption rates of O_2, CO_2 and H_2 were reduced in those experiments which involved circulatory disturbance (compare with table 2). The statement is often made in clinical literature that the meteorism of the intestine occurring in diseases which involve circulatory disturbance,—advanced heart disease, for example, cirrhosis of the liver, etc.,—is due to poor absorption of gas owing to interference with splanchnic circulation. As far as can be determined, there is little or no pertinent experimental work to support this theory. Experimental work such as that done by Kato (1910), who reported a slow absorption of CO_2 after ligation of the portal vein, or our present experiments, involve too gross a disturbance of the circulation to afford a useful comparison with clinical conditions; unless it be with those found in types of intestinal obstruction involving interference with the blood supply, or in certain cases of thrombosis of the mesenteric veins.

If the venous supply is obstructed without the introduction of a gas into the intestinal canal, a fluid exudate appears, probably due in part to increased permeability of the capillaries resulting from poor O_2 supply. This conclusion is borne out by several of our experiments in which a fluid dis-

tention of the entire small intestine occurred when the animal was breathing from a spirometer containing a low percentage of O_2.

SUMMARY

A study of the behavior of CO_2 in the stomach, as a typical case of gaseous exchange, results in strong evidence that the movement of this gas into and out of the stomach is not due to secretory factors but is governed by the physical laws of diffusion.

The behavior of O_2, N_2, H_2, CH_4, H_2S, CO_2 and air, when introduced into the gastro-intestinal tract, shows striking differences, ranging from the rapid disappearance of CO_2 to the relatively slight absorption of N_2 even after the lapse of a number of hours. The factors governing these differences are discussed; especially with reference to the laws of diffusion as defined by Exner. Partial obstruction of the portal vein causes a marked rise in CO_2 percentage and a corresponding increase in the total volume of gas in an intestinal loop.

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ON THE NATURE OF INHIBITION IN PENNATULA

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It is a commonplace of physiology that inhibition is the key to the problem of coördinated movement. And movement with economy of muscular action is displayed by worms, echinoderms, coelenterates. Meltzer (1899) considered the phenomena of inhibition coextensive in living matter with those of excitation. Keith Lucas (1917) has suggested that central inhibition may be of the Wedensky type, and Bruecke (1922) has demonstrated "apparent" inhibition in the central nervous systems of frog and cat. Veach (1925) and Veach and Pereira (1925) conclude that vagus inhibition of the movements of stomach and esophagus is a case of Wedensky inhibition. Howell (1925), on the other hand, in his review of the evidence, holds that special structures are essential to inhibition. He observes, however, that there is very little information on the nature of inhibitory and excitatory processes.

So far all studies of inhibition of this type have had to do with impulses travelling toward the effector in the same direction. No previous attempt has been made to analyze inhibition which results from a collision of two impulses. It has seemed to me, therefore, that certain facts noted in connection with the excitation and inhibition of luminescence in *Pennatula phosphorea* are of interest and significance. This sea pen is a feather-shaped colonial alcyonarian which when stimulated in the dark shows a luminescence composed of discreet points of light such as described by Parker (1920) for *Renilla*. The luminescence is not evenly distributed but moves from the point of stimulation in waves of light alternating with bands of darkness. Panceri (1872) has described and figured the course of these waves as they proceed away from the point of stimulation, be that where it may. Although it is possible to stimulate this form with induction shocks, it is better to use mechanical stimulation applied with a glass rod or simply with the finger, since less injury to the animal occurs in this way.

While it is true that the waves of luminescence proceed from any point

[1] Occupant of the Table of the American Association for the Advancement of Science, Naples, 1925.

touched, I found in some specimens that the waves passed more readily up the stem than down. For example, in one specimen, with a certain degree of stimulation applied at the base, eleven waves ascend, but if the same amount of stimulation is applied at the tip only three waves descend the stem. Ordinarily if a sensitive pen be stimulated at the base of the stalk, luminescent waves ascend to the tip as long as the stimulation (gentle pressure) is continued. If, at another time, excitation is applied at the tip a similar series of luminescent waves runs to the base. If tip and base of the pen are stimulated simultaneously, the waves from the two points of origin meet at the center and extinguish each other,—a case of inhibition by collision (Panceri, 1872).

I found that a striking demonstration of inhibition could be obtained in the following way. While the waves are running from base to tip as a result of gentle mechanical stimulation, if the tip of the colony then be stimulated by pressure of the finger, an area of darkness spreads from the tip to the base. Here are two seats of excitation but no luminescent waves are to be seen. Excitation at the tip has inhibited the effects of excitation at the base. If the exciting agent is withdrawn from one of the two loci then the waves of luminescence proceed from the remaining single point of stimulation as before. This experiment succeeds best with sensitive specimens freshly brought in and with the intensity of stimulation near threshold.

The inhibition at times may be incomplete. Thus, if a single source of excitation yields luminescent waves at the rate of 3 per second, simultaneous stimulation at the other end of the animal reduces the number to 1 per second, *i.e.*, the inhibition is partial.

As to the mechanism of this inhibition, it cannot be due to excitation of special inhibitory nerve fibers since solitary stimulation at one point always results in luminescent waves. The fact that luminescence in pennatulids appears in waves separated by dark areas clearly indicates that either the conducting system or the end organs have a refractory period following the excitation phase. If waves of excitation are set going from two points then sooner or later each phase of excitation must meet and fall within a refractory area. Here the excitation dies because it fails to be effective. If the refractory period were solely in the end organs then conduction would not be interrupted, and the wave of luminescence would appear on the other side of the dark band undiminished in intensity, i.e., the waves would meet and pass each other. This, however, is not the case. The refractory period is therefore in the conducting system. These facts mean that, in order to proceed, a nerve impulse must produce its effect in the conducting tissue at each point on the path of progress. If this reasoning is correct it necessarily implies the autocatalytic character of the impulse.

The question will at once be raised as to whether the phenomena described are of general occurrence in forms possessing the nerve net type of conduction. Two experiments with medusae, made long ago, would indicate an affirmative answer.

First, Bethe (1903) cites an experiment of Nagel's to the effect that he saw an inhibition reaction in medusa. If he touched the margin of the bell at one point, the manubrium responded by bending toward that point, then when he also touched the margin on the opposite side the manubrium fell down relaxed. In this case inhibition was caused by collision of impulses travelling to the manubrium from opposite sides, and the analysis I have made of inhibition in *Pennatula* would therefore apply here.

I was able to demonstrate a somewhat similar type of reaction in the Ctenophore, *Beroe forskalii*. Gentle mechanical stimulation on one side at the anterior end of the animal causes homolateral contraction of the longitudinal musculature so that the posterior end is curled upward and forward. If now while continuing the first stimulation weak mechanical excitation is exerted on the side opposite to the first, the "tail" not only straightens but relaxes. Here again impulses of approximately equal strength arrive from opposite directions and annul each other. The result is inhibition of muscular activity.

Another series of experiments made by Mayor (1908) and by Harvey (1911), on the pulsations of subumbrellar strips of the scyphomedusa (Cassiopea) shows a similar phenomenon. If, in this preparation, a single wave of contraction be started, it continues coursing around the ring for days. But if two waves of unequal magnitude are set going in opposite directions, then, upon colliding, the lesser is suppressed, while the greater continues. If two waves of equal magnitude are set off in opposite directions, upon colliding they both disappear; they mutually inhibit each other. Conduction of the impulse to muscular contraction in medusa therefore obeys the same law as the impulse to luminescence in *Pennatula*. Mayor considered the inhibition in this experiment to be referable to the refractory period of the musculature. Such an explanation cannot be correct since the same reasoning which we applied to the case of *Pennatula* would hold equally well here.

The facts described enable us to picture some of the forces at work in the conduction of an excitatory impulse. Since the phenomena of inhibition of the type under consideration take place in coelenterates where the conducting tissue is of the nerve net type, it is evident that special structures such as synapses and special inhibitory fibers are excluded from any part in the scheme. The inhibition is due entirely to the failure of the excitatory phase to pass a point in the conducting tissue which is for the moment in the refractory phase. It follows then that the reaction, which is the excitation wave, must involve successively every point in the path traversed. If we consider three consecutive points in the conducting

fiber, m, n and o, then the impulse cannot pass from m to o without involving n; o can only receive its impulse from n. It remains to be determined how small, as to space and time, these units have to be. Since the bands of darkness which occur between the waves of light in *Pennatula* are 3 to 5 mm. in width and the rate of propagation is approximately 50 mm. per second at 15°C., the duration of the refractory period lies between $\frac{1}{10}$ and $\frac{1}{20}$ second, assuming the band of darkness to be a measure of the extent of the refractory period of the conducting system. It follows, therefore, in *Pennatula*, that a delay of $\frac{1}{10}$ second in the propagation of the excitatory impulse is sufficient to extinguish it. Or, considered spatially, the excitatory wave is unable to "jump" over 3 to 5 mm. of inactive conducting tissue.

Inhibition of this type is similar to Wedensky inhibition in that each depends upon the excitatory impulse falling into a refractory phase of the conducting tissue. The two types differ because in the Wedensky phenomenon the excitatory impulses travel in the same direction and are not extinguished although they travel along conducting tissue which is in a refractory state, whereas inhibition by collision depends upon the excitatory impulses meeting head on, and as a result the extinction of each impulse in the area of tissue which is in the refractory phase.

CONCLUSIONS

Inhibition by collision of impulses in the nerve net system is referred to the extinction of the excitatory impulse when it falls into a refractory period. In order to proceed the impulse must be effective from point to point in the conducting tissue.

In *Pennatula* a refractory period of $\frac{1}{10}$ to $\frac{1}{20}$ second is sufficient to extinguish an excitatory impulse. Spatially this is equivalent to a distance of 3 to 5 mm.

The suggestion is made that a mechanism of this sort would account for the inhibition observed by Bethe in the manubrium of *medusa*, and the inhibition by collision described by Mayor and by Harvey for the muscular excitation of *Cassiopea*.

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THE PERMEABILITY OF PROTOPLASM TO IONS[1]

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In a recent article Osterhout (1) states that only undissociated molecules of H_2S penetrate the sap of *Valonia*, and he advances the hypothesis that only undissociated molecules can enter living protoplasm. We have an opportunity to test this in the case of arsenic and arsenious acids which were studied by the writer and reported some time ago (2). If Osterhout's generalization is justified it should hold for other substances than H_2S[2] and we should expect the rate at which arsenic enters the protoplasm and cell sap of *Valonia* to be proportional to the concentration of undissociated arsenic or arsenious acids in the solution to which the cells were exposed. How far this is true will be evident from what follows.

This work of the writer on arsenic was done with the species of *Valonia* (*V. macrophysa*) used by Osterhout and the plants were collected at the same place in Bermuda, so that variations due to difference in material may be excluded.

The experiments on arsenic were done by direct chemical analyses for arsenic in the sap and protoplasm after the plants had been allowed to remain in the solution containing arsenic for a definite length of time. Fortunately, it is possible by the Gutzeit method, which was used in this work and in an earlier work on *Nitella* (3), to determine accurately such small quantities as one micromilligram of arsenic. The arsenic compounds in the external solution were present in a concentration of 0.002 M. The concentration of ionized and unionized arsenic or arsenious acids was varied by the addition of NaOH to the dissolved acids so as to give solutions ranging from pH 5.0 to pH 9.0. Since the sodium salts of any acid are to be regarded as strong electrolytes they may be regarded as completely dissociated in the concentration used, thus simplifying the calculation of the proportion of the arsenic present in the ionic or molecular condition.

The argument of this paper is based upon rates of penetration, rather

[1] Approved for publication by the Surgeon General.

[2] As a matter of fact 2, 6, dibromo phenol indophenol appears to follow the same rules as H_2S. This will be published by the writer in the near future.

than upon equilibrium conditions. The entry of arsenic into the cells appears to be an orderly process whose essential nature is the same at the different H ion concentrations. Therefore the relative rates of penetration will be the same as the relative equilibrium concentrations, and either basis of comparison will lead to the same conclusion. This is the method used, for example, by Irwin (4) (under Osterhout's direction) in her work on dye penetration.

TABLE 1

Showing gram mols per liter of arsenic in the sap and protoplasm during indicated number of hours when Valonia had been placed in a solution of As_2O_3 or As_2O_5 in sea water at various pH values

pH	SAP				PROTOPLASM			
	1 hour	5 hours	10 hours	22 hours	1 hour	5 hours	10 hours	22 hours
	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$
				As_2O_3				
5.0	0.6	3.46	3.9	4.8	1.6	1.6	1.6	1.6
6.0	0.8	3.2	3.6	4.6	1.3	1.5	1.6	1.6
6.4	0.73	3.0	3.5	4.4	1.1	1.5	1.5	1.5
7.0	0.87	2.6	3.2	4.0	1.0	1.3	1.3	1.5
7.5	0.67	2.0	3.0	3.6	0.8	1.1	2.0	2.0
8.0	0.6	2.3	2.9	4.3	1.1	1.3	2.5	3.3
8.4	1.07	2.3	3.0	4.6	1 3	1.6	3.2	3.3
9.0	1.8	2.6	3.0	4.6	1.5	1.8	3.2	4.0
				As_2O_5				
	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$
5.0	4.6	5.3	11.0	16.0	1.66	2.3	4.2	4.5
6.0	4.2	5.3	10.0	12.0	1.6	1.66	2.8	3.2
6.4	2.2	4.8	7.3	7.3	0.87	1.2	1.66	2.0
7.0	2.2	3.8	6.4	6.2	0.87	1.2	1.5	1.6
7.5	1.0	4.8	4.6	4.4	0.8	1.5	2.2	1.4
8.0	2.0	3.2	4.2	4.6	1.66	2.3	3.0	3.2
8.4	4.0	5.3	6.0	6.7	2.0	2.3	3.3	3.6
9.0	4.0	5.3	6 7	8.0	2.2	2.6	3.8	3.8

The data which are used in this discussion are recalculated from tables in the previously published work (2) and are summed up in table 1. This shows the observed molecular concentrations of arsenic and arsenious acids respectively in the sap and the protoplasm at the end of one hour at different pH values of the external solution. The volume of the protoplasm can not be measured exactly in individual cases, as was stated in a previous paper (2), so the figures given are calculated on the basis of the observed average ratio of the weight of sap to protoplasm which was 25.3. Table

2 shows the molecular concentration of undissociated arsenic or arsenious acid and arsenate or arsenite ions respectively in the external solution at various pH values.

In comparing these two tables, it can be readily seen that the order of magnitude of the concentration of arsenic in the sap and the protoplasm remains the same when the H ion concentration in the external solution is varied. The order of magnitude of the concentration of dissociated arsenic in the external solution also remains the same at different pH values, while that of undissociated molecules changes with each change in pH value.

Is it more reasonable, in this case, to conclude that the penetration of arsenic depends upon the concentration of ions in the external solution or upon that of the undissociated molecules? That there are unknown factors involved is very evident, but these data certainly do not substantiate the hypothesis that only undissociated molecules can enter living protoplasm.

TABLE 2

Showing M concentration of undissociated molecules and ions in the external solution at indicated pH values

pH	AS MOLECULE IN EXTERNAL SOLUTION	AS ION IN EXTERNAL SOLUTION
	As_2O_3	
5	1.26×10^{-3}	0.74×10^{-3}
6	3.0×10^{-4}	1.7×10^{-4}
7	4.0×10^{-5}	$1.96+ \times 10^{-3}$
8	2.0×10^{-5}	$1.99+ \times 10^{-3}$
9	3.4×10^{-7}	$1.99+ \times 10^{-3}$
	As_2O_5	
5	4×10^{-6}	$1.99+ \times 10^{-3}$
6	4×10^{-7}	$1.99+ \times 10^{-3}$
7	4×10^{-8}	$1.99+ \times 10^{-3}$
8	4×10^{-9}	$1.99+ \times 10^{-3}$
9	4×10^{-10}	$1.99+ \times 10^{-3}$

Let us consider the data somewhat more in detail. The proportion of arsenic acid which is undissociated at pH 5 is 0.2 per cent. If the external solution contains arsenic acid in a concentration of 0.002 M at pH 5, the concentration of undissociated acid would be 0.000004 M. When cells of *Valonia* were exposed to such a solution, 0.002 M arsenic acid at pH 5, the concentration of arsenic acid in the sap at the end of one hour was slightly greater than that of unionized arsenic acid outside, while the concentration of arsenic acid in the protoplasm at this time was forty times greater than the concentration of the undissociated molecules in the external solution.

At pH 9 the concentration of arsenic in the sap is about 20,000 times greater than the concentration of undissociated molecules in the external solution. Furthermore, in the protoplasm, the concentration of arsenic acid at the end of one hour is 1,100,000 times greater than the concentration of undissociated molecules in the external solution.

When arsenious acid was used, the amount of undissociated acid at pH 5 was 63 per cent. The molecular concentration of acid used being 0.002, the concentration of undissociated acid was 0.00126 M. After the *Valonia* cells had been one hour in 0.002 M arsenious acid solution at pH 5 the sap contained only 0.000006 gram mols of arsenite per liter. Even after 22 hours in the solution when injury has set in, the concentration of arsenic in the sap is only 0.000048 gram mols per liter; this is only about $\frac{1}{1\,8\,5}$ of the concentration of undissociated molecules in the external solution. Meanwhile the concentration in the protoplasm is about $\frac{1}{1\,0}$ of that in the external solution.

At pH 9 the concentration of arsenious acid in the sap at the end of the first hour is 53 times greater than that of undissociated acid present in the external solution, while in the protoplasm it is 500 times greater.

It may be objected that the plants are injured by arsenic. Criteria of injury differ. Osterhout states: "At the end of each experiment all the cells that had been exposed to H_2S but which had not been pricked for the collection of sap were replaced in sea water and allowed to stand *overnight*"[3] and further, "It was observed that after an exposure of 2 hours to H_2S there were often signs of injury the next day." He supposes that the cells upon which he experimented were not sufficiently injured to affect the results.

The writer was careful to guard against this source of error by noting the length of time treated plants survived in sea water alone as compared with control plants. Normal plants survive at least 2 months in the laboratory when the water is changed every day; injury, if present, manifests itself during the first month. Obvious signs of injury, such as granulated or striated appearance of the protoplasm, change in color, firmness, etc., are always accompanied by cytolysis in a few days and are unmistakable evidences that the plant is not normal. Therefore if plants had not cytolyzed during the first month it was considered safe to consider them uninjured. Now, it was found that plants which had been in any of the arsenic solutions for even ten hours survived for a month at least. It seems to be an almost unavoidable conclusion that the plants used by the writer were very much less injured by the experiment than those used by Osterhout, and that if either set of data is to be discounted because of suspected injury it must be that presented by Osterhout. Had Osterhout observed his plants longer, he would no doubt, have found them cytolyzed in a very

[3] Italics mine.

few days. In this connection, it must be stated that his criterion of injury, the presence of SO_4 in the sap is evidently not a sufficiently refined criterion, for he states that no SO_4 was observed during the period of the experiment. Evidently the entrance of SO_4 is delayed and cannot be used as a criterion for injury except in the later stages of dissolution.

The data on both protoplasm and sap show a minimum rate of penetration of arsenic from solutions near neutrality. There are therefore other factors involved besides dissociation, but in their consistent order of magnitude the internal arsenic concentrations at various pH values are in much better agreement with the ionic concentrations than with those of the undissociated molecules in the external solution.

The most important point shown by the table is that the rate of penetration of arsenic into either the sap or the protoplasm is very far from being proportional to the concentration of undissociated acid, but that there is a relatively constant proportionality between the rate of penetration of arsenic and the concentration of arsenate or arsenite ions in the external solution.

Table 1 also shows that the concentrations of arsenic in the protoplasm were of a higher order of magnitude than those in the sap, but much below those of the external solution. Except in the case of arsenite solutions, where injury quite possibly affects the last observations, the penetration of arsenic seems to have nearly or quite reached equilibrium within 22 hours. This leads us to the conclusion that in normal cells the arsenic concentrations of the protoplasm and sap never equal one another, nor does the arsenic concentration in the protoplasm ever become equal to that in the external solution unless death sets in. Therefore, one may not wisely deduce the course of events in the protoplasm from observations on sap alone. After all, the sap must be regarded as a substance secreted by the protoplasm, and conclusions concerning the protoplasm should be substantiated by direct experimentation upon the protoplasm itself, otherwise such results are open to the same criticisms which confront other indirect methods.

SUMMARY

The studies on penetration of arsenic into the sap and protoplasm of *Valonia* do not support the hypothesis of Osterhout that only undissociated molecules enter living protoplasm.

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AN EXPERIMENTAL STUDY OF TONUS IN SKELETAL MUSCLES AS RELATED TO THE SYMPATHETIC NERVOUS SYSTEM

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Unmyelinated nerve-fibers of small caliber which are morphologically identical with sympathetic fibers and terminate on skeletal muscle-fibers have previously been described. However, the experimental anatomical studies of Boeke (1913) afford the first complete anatomical demonstration of the sympathetic innervation of skeletal muscles. Boeke's findings are corroborated by the results of later experimental anatomical studies of Boeke and Dusser de Barenne (1919), Agduhr (1919) and a number of other investigators, including the present writers (1924). The sympathetic innervation of skeletal muscles may, therefore, be regarded as an anatomical fact.

While certain physiological studies yielded only negative results, others seem to indicate clearly that the sympathetic nervous system exerts an influence in the functional control of skeletal muscles. However, there has been no general agreement regarding the quality or the functional significance of this influence.

The importance of the sympathetic innervation of skeletal muscles as a clinical as well as a laboratory problem has been emphasized recently by Hunter (1924) and Royle (1924). These investigators have vigorously supported the theory set forth by Langelaan (1915), according to which the plastic tonus of skeletal muscles is maintained through the sympathetic and their contractile tonus through the somatic nervous mechanism. They define postural tonus as a combination of plastic and contractile tonus which together are responsible for the posture imposed on skeletal muscles. They are also of the opinion that conditions of hypertonus, or spasticity, in skeletal muscles, in the presence of an impaired voluntary nervous mechanism, are brought about by influences exerted through the sympathetic nervous system. If the impaired voluntary mechanism can not overcome this spasticity, the muscles become functionally useless. Assuming that the spasticity is brought about through the sympathetic nervous system, they reasoned that if the voluntary nervous mechanism involved is not completely destroyed it might, in the absence of sympa-

thetic influences, regain functional control of such a group of muscles. Accordingly, they recommended, for selected clinical cases, the surgical procedure known as sympathetic ramisection, the aim of which is the elimination of the sympathetic nerve supply to the muscles in question. This procedure has, in their hands, yielded gratifying results. The results reported by certain other investigators, notably Kanavel, Pollock and Davis (1924), are less encouraging.

Obviously, no clinical procedure of such drastic character should find general application until the underlying principles on which it is based are fully established. Hunter and Royle, with their associates, have made extensive investigations on laboratory animals as well as clinical patients and have drawn conclusions which have profoundly influenced the attitude both of practitioners and laboratory investigators who are interested in these problems. However, the experimental facts available are not regarded by all as sufficient to justify such far-reaching conclusions. Consequently, there is urgent need of further investigation of a more intensive character.

The present paper embodies the results of a series of experimental studies in which an attempt was made to control the conditions of the experiments as far as possible, and to check the results both by qualitative and quantitative methods.

REVIEW OF LITERATURE. The literature bearing on the rôle of the sympathetic nervous system in the production and maintenance of tonus in skeletal muscles has been reviewed recently in a number of papers, notably those of Spiegel (1923), Riesser (1925), and Cobb (1925). Therefore, we shall refer briefly only to those papers which bear directly on those phases of the problem which are involved in the present investigation.

The earliest investigators in this field quite logically surmised that whatever influence is exerted on skeletal muscles through the sympathetic nervous system must be related to tonus. As a result of his early experimental studies on frogs and cats, de Boer (1913) advanced the theory that the tonus of skeletal muscles is mediated solely through the sympathetic nervous system. Although this theory has not been supported by the findings of other investigators, it focussed attention on muscle tonus as related to the sympathetic nervous system.

Negrin y Lopez and von Brücke (1916) and Dusser de Barenne (1916) reported an initial hypotonus in the muscles of the corresponding hind limb in cats, following unilateral extirpation of the lumbar sympathetic trunk, which gradually subsided. Cobb (1918), using cats, and Takahashi (1922), using guinea pigs as the experimental animals, could not demonstrate to their own satisfaction even an initial hypotonus in the muscles of the hind limbs following elimination of their sympathetic

innervation. Salek and Weitbrecht (1920) could not invariably demonstrate a reduction in the tonus of the muscles of the hind limbs of frogs following elimination of their sympathetic nerve supply. Maumary (1922), who studied the muscles of the hind limbs of frogs following unilateral and bilateral section of the corresponding communicating rami, expressed the opinion that the tonus of skeletal muscles depends on the sympathetic nervous system, but that there is in addition to the sympathetic tonus a labyrinthan tonus of spinal origin. More recently Newton (1924) observed, in rabbits, no reduction in the tonus of the muscles of the hind limbs following elimination of their sympathetic nerve supply.

The characteristic posture of the limbs of mammals, due to spastic contraction of the extensor muscles in the state of decerebrate rigidity, is well known. If tonus is maintained solely or in part through the sympathetic nervous system, we should expect that elimination of the sympathetic nerve supply to a limb might either prevent the onset of decerebrate rigidity in that limb or bring about a material reduction in the degree of rigidity exhibited by the muscles. However, studies in decerebrate rigidity in animals, in which one or more limbs were deprived of their sympathetic innervation, have afforded little evidence of real value. Nor have the results of such studies by different investigators been at all constant. Dusser de Barenne (1916) reported reduction in the rigidity in some, but not in all cases. Van Rijnberk (1917) and Cobb (1918) failed to observe any effect of sympathetic extirpation on decerebrate rigidity. In summarizing the evidence available in 1920, Adrian says "it appears, then, that although removal of the abdominal sympathetic chain is occasionally followed by a diminution of the tone on that side, yet in the majority of these experiments the loss of the sympathetic supply makes no difference; the rigidity is developed and maintained as well on the operated as on the intact side."

Royle (1924) has recently emphasized the advisability of performing sympathectomy some time before the decerebration experiment is carried out. By this method, any sensory disturbances that might be set up by the wound caused by sympathectomy are avoided. He also employed intratracheal anesthesia before decerebration and pumped fresh air into the lungs afterwards. He says that "in all these experiments the onset of decerebrate rigidity was characterized by extension of all four limbs, but the left lower limb could be much more easily relaxed than the right. In the intervals, when the rigidity of the lower limbs was less, it was seen that the characteristics of decerebrate rigidity, as described by Sherrington (1896), were not present. If the right lower limb, for example, were passively extended, the limb tended to remain in extension; if the left one were extended, the limb would fall into a flexed position as soon as it was released."

Still more recently, Kanavel, Pollock and Davis (1924) reported only negative results of a series of experiments in which they attempted to demonstrate any possible differences in the extensor rigidity of the two hind limbs in a series of cats subjected to decerebration following unilateral extirpation of the lumbar sympathetic trunk. Meek and Crawford (1925) studied the knee jerk in a series of twelve dogs subjected to decerebration following unilateral lumbar ramisection in nine, and unilateral extirpation of the lumbar sympathetic trunk in three of the animals. They found that "the 'shortening reaction' of Sherrington appeared in all cases on the operated as well as the unoperated side." They concluded, therefore, that "in the dog, plastic tonus does not depend on sympathetic innervation." Likewise, Huggett and Mellanby (1925) who studied "the influence of the sympathetic, parasympathetic and somatic systems of nerves on the tonus of muscle in the intact and decerebrate cat" by means of drugs were unable to demonstrate any tonic influence either of the sympathetic or parasympathetic systems, but found that "the intravenous injection of curare (5 mgm.) into a decerebrate cat results in the immediate disappearance of all rigidity and the production of complete flaccidity of the muscles." "This quantity of curare," they say, "does not paralyse the sympathetic system of nerves since a typical adrenalin rise of blood pressure may be produced in a completely curarised decerebrate cat under artificial respiration." They assume, therefore, that the disappearance of rigidity is due to paralysis of the somatic motor nerve endings and conclude that "the normal tonus of muscle and the exaggerated postural tonus of the muscles of the decerebrate cat are the immediate result of impulses passing to the voluntary muscle through the somatic system of nerves." On the other hand, Coombs and Tulgan (1925) observed, in a number of decerebration experiments on cats, that "the rigidity of the fore limbs was very much diminished while the rigidity of the hind limbs persisted unchanged," following extirpation of the stellate ganglia.

Hunter (1924) found birds more favorable, in certain respects, than mammals for experimental study of the influence of the sympathetic innervation of a limb on the postural tonus of its muscles. The effect of removal of the sympathetic innervation is not obscured, in the resting wing, by the contractile mechanism, as is the case in the mammalian limb. Following section of the sympathetic trunk, in the fowl, immediately caudal to the roots of the nerves which make up the brachial plexus, i.e., just above the first thoracic nerve, he found that the adducted position characteristic of the wing at rest was no longer fully maintained. Section of the dorsal root of the first thoracic nerve produced precisely the same effect. The visceral afferent and preganglionic efferent neurons whose fibers enter the cervical sympathetic trunk are components of the upper thoracic nerves. Therefore, Hunter interpreted the results of these

experiments as indicating that the plastic tonus of the wing muscles is maintained through the sympathetic system by a system of proprioceptive reflex arcs whose afferent and efferent limbs are components of the upper thoracic nerves. The afferent and efferent limbs of the somatic reflex arcs which subserve the contractile tonus of the wing muscles are components of the lower four cervical nerves. Following section of the dorsal roots of these nerves, the wing no longer tends to take up the usual folded position, but tends to remain in any position in which it is passively placed. If, following section of the dorsal roots of the brachial nerves, the sympathetic trunk also is cut just above the first thoracic nerve, the wing no longer tends to remain in any position in which it is passively placed, but hangs dependent. According to Hunter, "this is conclusive proof that plastic tone is subserved by the sympathetic innervation of the skeletal muscles of the wing." Analogous results were obtained in sea gulls by the same surgical procedures.

The results of these experiments show clearly that when the wing is deprived only of its sympathetic innervation, the tonus of the muscle is reduced so that the normal posture is not perfectly maintained. However, as observed by Hunter, "considerable tone remains, and it tends to impose a folded posture on the wing. This influence must be due to the presence of the somatic proprioceptive reflex arcs which remain intact, but in the absence of the influence of the sympathetic innervation of the wing, the maximum position of adduction can no longer be maintained against the force of gravity. This force leads to some degree of drooping and abduction of the wing. During light anesthesia the effect is rendered more evident as the result of the partial removal of the influence of the somatic proprioceptive connections." He further stated that the influence of the somatic arcs is also markedly reduced by fatigue which, if carried far enough, may leave the wing in a condition which is not far removed from flaccid paralysis.

EXPERIMENTAL PART. *I. Preliminary experiments on dogs. a. Procedure.* In our earliest experiments we adopted the plan of extirpating the sympathetic trunk in the lumbar region on one side only, using dogs as the experimental animals, and comparing the tonus, the power of contraction, and the resistance to fatigue of the muscles of both hind limbs, both immediately after the operation and after ample time had been allowed for the degeneration of the sympathetic nerve-fibers supplying the muscles of the hind limb on the side of the operation. More than twenty dogs were used in this series of experiments. In a smaller number of dogs the inferior cervical ganglion was extirpated on one side, and the muscles of the forelimbs were studied by the same comparative methods.

All operative procedures were carried out under ether anesthesia and, in all cases in which the animals were allowed to live following operation,

aseptic precautions were observed. The sympathetic trunk, usually on the left side, was regularly extirpated from the fourth to the seventh lumbar segments inclusive. This operation effectively eliminates the sympathetic nerve supply to the hind limb, with the exception of those fibers which run down along the femoral artery from the aortic plexus. In some instances the first sacral segment of the sympathetic trunk was also removed. This, as is also indicated by the results, is unimportant since section of the sympathetic trunk in the lower lumbar levels severs all preganglionic fibers to the first sacral sympathetic ganglion. Extirpation of the inferior cervical sympathetic ganglion eliminates the entire sympathetic supply to the forelimb, since all preganglionic fibers to the cervical sympathetic ganglia are components of the upper thoracic nerves.

The animals which were allowed to live following sympathetic extirpation were kept for varying intervals up to three and one-half months. During this time they were kept under observation and at intervals subjected to various tests designed to reveal any differences which might be exhibited by the corresponding muscles of the two limbs.

b. Results. In the early part of this work we were quite convinced that some of the animals showed a degree of hypotonus, which could be detected by palpation for some time following operation, in the muscles of the limb deprived of its sympathetic innervation. This observation was reported in a preliminary communication (1924) as corroborating the observations of Negrin y Lopez and von Brücke (1916) and Dusser de Barenne (1916) on cats. Throughout the course of our experiments we have had many animals (dogs and cats) in which we were unable by palpation to detect even an initial hypotonus in the muscles of the corresponding limb following sympathetic extirpation. Therefore, we do not now regard this observation as of material importance. We have more recently demonstrated by more exact methods, which will be outlined in a later section of this paper, that the tonus of skeletal muscles is reduced following elimination of their sympathetic nerve supply, although this may not be detectable by palpation or direct observation. None of our dogs showed any impairment of function or weakness of the muscles deprived of their sympathetic innervation which we were able to detect in the normal behavior of the animals. In the absence of graphic records, the tendon reflexes also were adjudged to be normal.

When these animals were subjected to surgical anesthesia, a marked difference in the flaccidity of the muscles of the two limbs became apparent. As the animals, which had previously been subjected to extirpation of the left lumbar sympathetic trunk, lay on their backs, the left hind limb dropped to a lower level than the right. The left thigh was also abducted to a greater degree than the right. The characteristic postures of the hind limbs under these conditions are illustrated photographically in

figure 1. When equal weights of 100 or 200 grams were attached to both hind feet and suspended over pulleys at the end of the table, the left limb was more fully extended than the right (fig. 2). When equal weights of 600 grams or over were attached to the feet, both limbs were drawn out equally, both being fully extended. In the case of animals which had been subjected to extirpation of the left inferior cervical ganglion, the left forelimb dropped to a lower level than the right. The left arm was slightly abducted, and the angle between the arm and forearm was less on the left side than on the right. The characteristic posture of the forelimbs under these conditions is illustrated photographically in figure 3.

The experiments described above were repeated in some of our animals at intervals of a week or longer with uniformly constant results. It should be noted, however, that in two animals in this series no marked differences in the flaccidity of the muscles of the two hind limbs were observed even under anesthesia. In both these animals post-mortem examination showed that the left lumbar sympathetic trunk had been successfully extirpated. These animals constitute exceptions for which we have no explanation. Another dog which was subjected to extirpation of the left lumbar sympathetic trunk when about two months of age showed the usual difference in the flaccidity of the muscles and the posture of the right and left hind limbs, while under surgical anesthesia, ten weeks after the operation. These differences did not appear when the animal was again subjected to surgical anesthesia five weeks later. Neither did loads of 100 or 200 grams attached to the feet, at this time, draw out the left leg farther than the right, but drew out both legs equally.

During the administration of ether, as the anesthesia approaches the surgical stage, there is a brief interval of excitation during which the normal limbs exhibit extensor rigidity to a marked degree. Such extensor rigidity was usually greatly reduced, in our animals, in the limb which was deprived of its sympathetic innervation. Inasmuch as general anesthesia affects first the higher cerebral centers and then the centers in the brain stem, this extensor rigidity is probably akin to decerebrate rigidity. This observation is, therefore, in accord with the results of those decerebration experiments cited above in which extensor rigidity was less marked in the limb deprived of its sympathetic innervation than in the opposite limb with its sympathetic nerve supply intact.

During recovery from anesthesia, as the animals began to use their limbs, the limb on the unoperated side was usually used earlier and more effectively than the one deprived of its sympathetic innervation. As the animal attempted to stand, it would not uncommonly fall toward the side of the operation more frequently than toward the opposite side. Even after it was able to walk, the limb deprived of its sympathetic innervation

was used awkwardly and was apparently lacking in strength until the animal had to a considerable degree recovered from the effects of the anesthetic.

By the procedure outlined above, a reduction in the tonus of skeletal muscles, following elimination of their sympathetic nerve supply, which can not be detected by direct observation or palpation while the animal is in the waking state, is revealed while the animal is under anesthesia. This suggests that the somatic innervation in large measure compensates for the loss of the sympathetic innervation of the muscles in the maintenance of normal posture, and that the somatic nervous mechanism is more profoundly affected by anesthesia than is the sympathetic. The latter supposition is in full accord with the common observation that smooth muscle, e.g., the gastro-intestinal musculature, is not relieved of nervous control during anesthesia.

The experimental results set forth above strongly suggest that the sympathetic innervation is a factor in the tonus of skeletal muscles. However, the absence of this factor may be quite completely obscured, under ordinary conditions, by the compensatory effect of the somatic proprioceptive mechanism. Herein, probably, lies the explanation of many of the recorded failures to recognize any influence of the sympathetic innervation on skeletal muscles.

II. Experiments on birds. The experiments on fowls and pigeons, here reported, are in the main a repetition of certain of Hunter's experiments on birds. The results obtained in this series of experiments in general corroborate his results. As pointed out by Hunter (1924), birds are more

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Explanation of figures 1 to 5

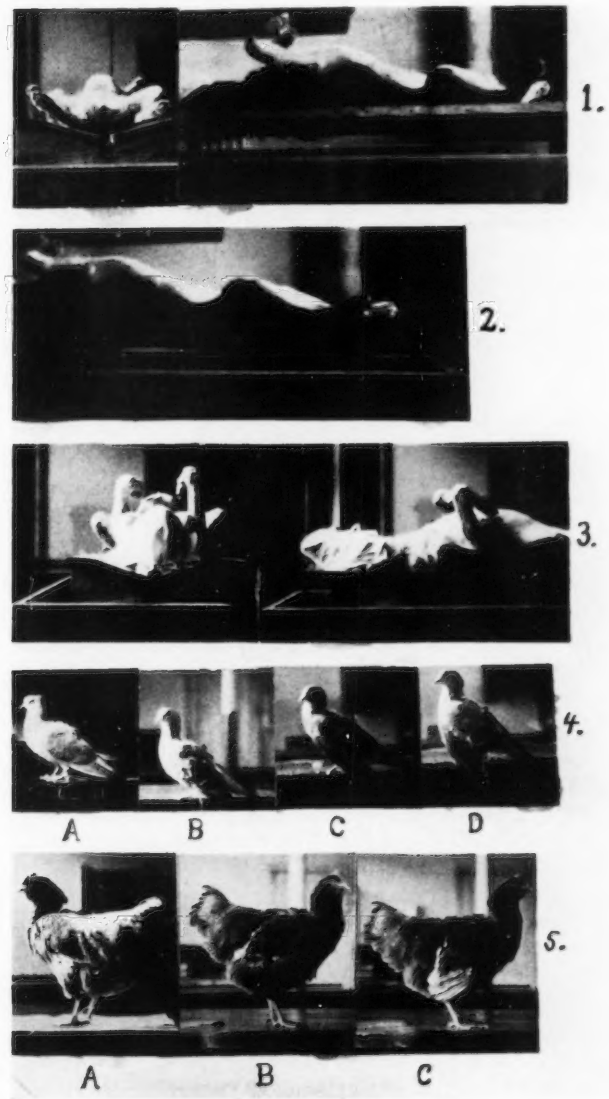
Fig. 1. Dog, three weeks after extirpation of the left lumbar sympathetic trunk, showing difference in posture of the right and left hind limbs while under anesthesia. The muscles of the left limb are more flaccid, the thigh is abducted to a greater degree, and the leg rests in a lower position than the right.

Fig. 2. Dog, same as in figure 1, showing greater extension of the left than of the right hind limb due to equal weights of 200 grams attached to both feet and suspended over pulleys.

Fig. 3. Dog, one month after extirpation of the left inferior cervical sympathetic ganglion, showing difference in posture of the right and left forelimbs while under anesthesia.

Fig. 4. Pigeons showing posture of left wing following operation. *A*, left sympathetic trunk cut just caudal to brachial plexus; *B*, dorsal roots of left brachial plexus cut, usual posture of the wing while at rest; *C*, dorsal roots of the left brachial plexus cut, wing drawn down remains in that position; *D*, same as *C*, wing drawn down still farther remains in that position.

Fig. 5. Fowl showing posture of wing following operation. *A*, left sympathetic trunk cut just caudal to brachial plexus. *B*, dorsal roots of right brachial plexus cut, wing molded into normal position. *C*, same as *B*, wing drawn down passively remains in that position.



Figs. 1 to 5

favorable than mammals for the study of the influence of the sympathetic nervous system on limb posture. The effect of removal of the sympathetic innervation is not obscured in the resting wing by the influence of the somatic nervous mechanism to the same extent as in the mammalian limb.

Section of the sympathetic trunk in birds just caudal to the brachial plexus, i.e., just above the first thoracic nerve, as practiced by Hunter, completely eliminates the sympathetic innervation of the wing by reason of the fact that the preganglionic fibers to the cervical sympathetic ganglia are components of the upper thoracic nerves. Both in fowls and pigeons, in which the sympathetic trunk was divided just caudal to the brachial plexus, we observed, as Hunter had reported, that the resting wing no longer maintained its characteristic folded and adducted position, but drooped slightly outward and downward as illustrated in figures 4, *A*, and 5, *A*. The birds still retained complete voluntary control of the wing and would occasionally replace it in its normal resting position, only to have it drop back again as soon as the contractile mechanism relaxed. As reported by Murray (1925), we also observed that the defect in the posture of the wing became less apparent in the course of a few weeks. This is probably due to the compensatory action of the somatic nervous mechanism. Murray found that the wing regains its normal resting position in about sixty days. None of our birds was kept alive until the normal posture of the wing had been fully restored.

Section of the dorsal roots of the nerves composing the brachial plexus, i.e., the lower four cervical nerves, interrupts the somatic reflex arcs to the wing. However, the proprioceptive reflex arcs whose afferent and efferent limbs join the brachial nerves via the cervical sympathetic rami remain intact. In birds subjected to this operation the normal tendency of the wing to take up the folded and adducted position was absent. When the wing was passively placed in its normal flexed position, it tended to remain there. When placed in any other position intermediate between the normal resting posture and the fully dependent position, it tended to remain in whatever position it was placed (figs. 4, *C* and *D* and 5, *C*). When, in these birds, the sympathetic trunk also was severed just caudal to the brachial plexus, the wing no longer tended to remain in the position in which it was passively placed, but hung dependent.

As pointed out by Hunter, the wing, following section of the dorsal roots of the brachial nerves, exhibits the properties of a plastic body; therefore, it tends to remain in any position in which it is placed. The flexor muscles exhibit the "shortening" and "lengthening" reactions which, according to the original conception of Sherrington, are manifestations of plastic tonus. The tendency of the wing to maintain its normal posture is absent by reason of the loss of contractile tonus, which, according to this view, is normally maintained through the somatic reflex arcs.

III. Experiments in measuring muscle tonus. a. Introductory remarks. The results of the experiments, both on mammals and birds, recorded above indicate clearly that the sympathetic nervous system plays an important rôle in the functional activities of skeletal muscles, especially in the maintenance of posture. They strongly suggest that an important component of muscle tonus is mediated through the sympathetic nervous system. Yet, in the absence of additional comparative data other than those which depend on sense impressions or are obtained by direct observation, they can hardly be regarded as convincing. Additional data which approach quantitative accuracy are urgently needed. The unreliability of direct observations and sense impressions in studies of this character is amply illustrated by the conflicting statements regarding the effect of unilateral sympathectomy on the muscles of the corresponding hind limb in frogs and mammals set forth in the review of the literature in an earlier section of this paper.

In seeking a method by which muscle tonus may be measured with some degree of accuracy, we found no other which seemed to fulfill the requirements of this investigation quite as well as the method set forth and employed by Spiegel (1923). He selected the quadriceps femoris as convenient for the purposes of his investigation because it is a postural muscle, and by reason of mechanical considerations. This muscle, being an extensor involved in the maintenance of the posture of the limb, also fulfills the requirements of the present investigation.

b. Outline of method. The apparatus used differs only in details from that used by Spiegel. The animal rests on a board clamped to the table. The pelvis projects beyond the end of the board just far enough to permit the hind limbs to hang down with the thighs in the vertical position. The apparatus (fig. 6) for measuring the tonus consists essentially of a narrow leg board, *LB*, with a transverse bar, *CB*, attached at its upper end, two lever rods, *LR*, attached to the ends of the transverse bar and bent at an angle of 135° with the leg board, and a hinged support, *F*, for the leg board, which may be clamped to a vertical rod, *VR*, extending downward from the board on which the animal rests. The support for the leg board consists of a curved fork, *F*, which is hinged with two rods, *R*, which are attached to the under surface of the leg board, so that they extend about 3 cm. from its upper end. These rods are bent so that the axis of rotation of the hinge, *H*, falls approximately 0.5 cm. from the plane of the under surface of the leg board. A protractor is attached at one end of the transverse bar so that its plane edge is parallel with the plane of the leg board. A weighted silk thread is suspended from the center of the protractor and a scale pan, *S*, for the reception of weights is suspended by strong cords from the distal ends of the lever rods. The animal's leg, which hangs between the rods extending from the upper end of the leg

board, is fixed to the under surface of the leg board, the end of which is concave to avoid pressure on the flexor tendons, in such a position that the axis of rotation of the knee joint coincides with the axis of rotation of the hinge. The apparatus is then adjusted so that the femur is in a vertical position when the pelvis is fixed and the quadriceps is not pressed against the board on which the animal rests. If the angle of the leg board with

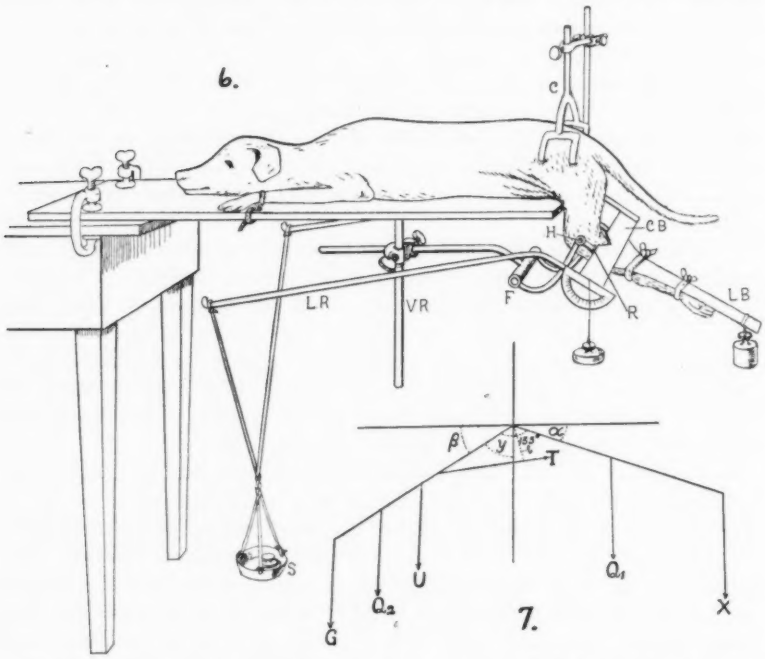


Fig. 6. Apparatus for measuring muscle tonus (modified from Spiegel). For explanation see text.

Fig. 7. Schematic diagram of forces acting in the use of the apparatus illustrated in figure 6 (after Spiegel). U = weight of leg and foot; Q_1 = weight of lever rods; Q_2 = weight of leg board; x = weight applied in the scale pan; G = weight applied to leg board to obtain desired angle; T = pull of the extensor tendon; t = distance of tendon from axis of rotation of the knee; y = angle of rotation as read on protractor.

the vertical is greater than 20° or 25°, sufficient weight may be attached to the lower end of the leg board to reduce this angle to 20° or less, as desired. The apparatus with the leg in place while at rest is a balanced system. Any weight now added to the scale pan will bring about some rotation of the hinge of the apparatus and corresponding flexion of the knee. The angle of rotation is read off on the protractor.

When cats were used as the experimental animals, we commonly added 10-gram weights in succession and recorded the angles of rotation as read off on the protractor. When dogs were used, we found 20-gram weights more convenient. If the animal remains quiet and refrains from voluntary movements of the hind limb, the experiment may be carried out without anesthesia. In most instances, however, it was necessary to place the animal under light anesthesia in order to avoid voluntary movements. We found, as Spiegel also reported, that light ether anesthesia does not affect the measurements. In the case of animals which would permit of carrying out the experiment in the waking state, we always obtained the same results with and without anesthesia.

The data necessary for the calculation of the tonus curve include the readings taken after the death of the animal. These were taken, with the leg in the apparatus as before, a little while after the death of the animal, but before the onset of rigor mortis.

Whenever the apparatus is balanced, the sum of the forces applied on one side of the axis of rotation, tending to rotate in one direction, is equal to the sum of the forces applied on the opposite side, tending to rotate in the opposite direction. This equality, in the case of the dead animal, is expressed by equation

$$\text{I} \qquad x'l_1 \cos\alpha + Q_1q_1 \cos\alpha = (Uu + Gl_2 + Q_2q_2) \cos\beta.$$

In this equation x' is the weight in the scale pan; Q_1, the weight of the lever rods, Q_2, the weight of the leg board; U, the weight of the leg and foot; G, the weight attached to the leg board to bring about the desired degree of extension of the knee; l_1, l_2, q_1, q_2, u respectively, the distances from the axis of rotation at which the respective weights are applied; α and β, the angles respectively of the lever rods and the leg board with the horizontal. In the case of the living animal equation I becomes equation

$$\text{II} \qquad x''l_1 \cos\alpha + Q_1q_1 \cos\alpha = (Uu + Gl_2 + Q_2q_2) \cos\beta + Tt.$$

In this equation T is the tension of the quadriceps; t, the distance of the tendon from the axis of rotation of the knee joint; x'', the weight in the scale pan. Equation II minus equation I is expressed as follows:

$$Tt = (x'' - x')l_1 \cos\alpha.$$

However, α is not measured and y is the angle of the leg board with the vertical.

$$\alpha + \beta + 135° = 180°, \text{ and } \beta = 90° - \text{y}. \text{ Therefore,}$$

$$T = (x'' - x') \cos (y - 45) \frac{l_1}{t}$$

In this equation y is the angle read on the protractor; x'', the weight in the scale pan; x', the weight in the scale pan necessary for the same value of

y in the case of the dead animal; l_1, the length of the lever rods; t, the distance of the extensor tendon from the axis of rotation of the knee for the corresponding position of the leg. The tonus curve is plotted by using the values of T, as calculated for the successive positions of the leg, as the abscissae and the angles of flexion as the ordinates.

c. Actual experiments. The tonus curve, i.e., the curve of passive extension, of the quadriceps muscle of the normal animal, obtained by the method outlined above, rises very slowly at first and then more rapidly as the angle of flexion increases (fig. 8). This indicates that the quadriceps muscle offers greater resistance at the beginning of passive displacement of the leg from a position of rest in which the knee is only slightly flexed than it does from one in which the angle of flexion is relatively great. That is, when the muscle is near its minimum normal length in the resting position it offers greater resistance to postural change than after its length is increased by passive displacement of the leg.

Apparently, this phenomenon is common to muscles which normally maintain posture against the force of gravity. It was recognized and described by Rieger (1906), who applied to it the term "Bremsung" (brake phenomenon). Spiegel (1923) also described it as characteristic of the quadriceps, under normal conditions, in man as well as in animals. He further pointed out its independence of the action of the antagonists. It is not affected by section of the flexor tendons, and still persists in preparations in which all the muscles of the thigh except the quadriceps are removed. On the other hand, it is not an inherent capacity of the muscle. It is absent in the dead animal. Neither is it exhibited by a denervated muscle. Spiegel also showed that the brake phenomenon is absent when the proprioceptive reflex arcs are interrupted by degeneration of the dorsal root fibers in certain pathological conditions, e.g., tabes dorsalis. On the other hand, it is greatly exaggerated in certain other pathological conditions, e.g., spastic hemiplegia. These facts indicate that it is determined and conditioned by the innervation of the muscle.

The brake phenomenon illustrates the capacity of the muscle, under normal innervation, to resist extension, while in a resting position, independently of the nervous processes which initiate and control active contractions. Therefore, it involves that component of tonus which Sherrington designated "plastic" tonus. If plastic tonus is mediated through the sympathetic nervous system, we might reasonably anticipate the absence of the brake phenomenon in muscles deprived of their sympathetic innervation. Accordingly, we undertook a series of experiments designed primarily to detect a possible relationship of the brake phenomenon to the sympathetic innervation and also to obtain comparative quantitative measurements of this type of muscle tonus before and after sympathetic extirpation.

More than twenty-five animals (cats and dogs) were used in this series of experiments. In some experiments the tonus of the quadriceps muscle was measured, according to the method outlined above, in the normal animal; then the lumbar sympathetic trunk was extirpated, under aseptic conditions, and after a few days or weeks the tonus of the quadriceps was measured again. The tonus curves obtained before and after operation were then compared. In other experiments the tonus curves of both quadriceps were obtained for comparison, the animal having previously been subjected to unilateral extirpation of the lumbar sympathetic trunk. Both procedures yielded identical results because the tonus curves of both quadriceps in normal animals are commonly practically identical.

The tonus curves of the right and left quadriceps respectively obtained in a dog eight days after extirpation of the left lumbar sympathetic trunk are illustrated in figure 9 *A*. The curve at the right, *R*, is the tonus curve of the normal right quadriceps. The curve at the left, *L*, is the tonus curve of the left quadriceps deprived of its sympathetic nerve supply. As indicated by the slow rise of the curve *R* at the beginning, the brake phenomenon was well developed in the normal right quadriceps until the angle of flexion of the knee reached approximately 30°, after which it was greatly reduced. As indicated by the immediate and continued rapid rise of the curve at the left, *L*, the brake phenomenon was absent in the left quadriceps deprived of its sympathetic nerve supply. Figure 9 *B* illustrates the tonus curves of the quadriceps muscles of a cat three days after extirpation of the left lumbar sympathetic trunk. In this instance also the brake phenomenon was well developed in the normal muscle and absent in the one deprived of its sympathetic nerve supply. These curves may be regarded as quite typical of the tonus curves of the quadriceps muscles, with and without their sympathetic nerve supply, obtained in a relatively large series of experiments.

The length of the interval between sympathetic extirpation and measurement of the muscle tonus is relatively unimportant. Curves obtained on the second and third days following operation are practically identical with those obtained two weeks or longer following extirpation of the lumbar sympathetic trunk. In two experiments tonus curves of the quadriceps muscles were obtained one hundred twelve days after unilateral extirpation of the lumbar sympathetic trunk. These curves (fig. 9, *C*) show essentially the same difference in the tonus of the muscles with and without their sympathetic nerve supply as those obtained within a few days following sympathetic extirpation. In some experiments measurements were carried out immediately after operation. In most of these the results were practically identical with those obtained later. In a few the quadriceps on the side of the operation showed a higher degree of tonus than the one on the unoperated side. This was probably due to stimulation caused by

the operation. When these animals were allowed to rest for several hours and the measurements were again taken, the curves obtained showed essentially the same difference in the tonus of the two quadriceps muscles as is indicated by the curves obtained several days after operation.

As indicated above, the tonus curves of the same muscle obtained before and after elimination of its sympathetic nerve supply are essentially identical with the tonus curves of the muscle on the side of the intact sympathetic supply and the one of the muscle deprived of its sympathetic nerve supply respectively which are obtained at the same time. Such inherent differences in tonus as might be present in the corresponding muscles of the two limbs in the normal animal apparently are not sufficient to vitiate the results of the experiment. In order to check the results still

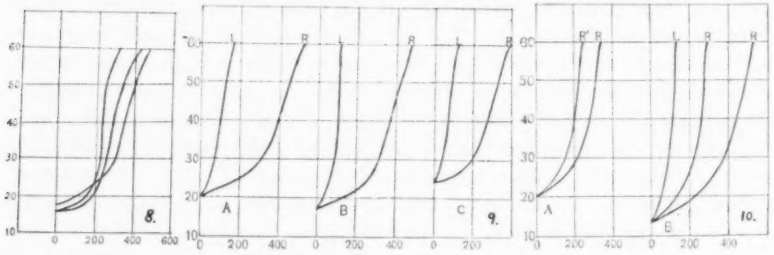


Fig. 8. Tonus curves of the normal quadriceps muscle in cats.

Fig. 9. Tonus curves of right, *R*, and left, *L*, quadriceps muscles following extirpation of the left lumbar sympathetic trunk.

A. Curves obtained from a dog 8 days after operation.

B. Curves obtained from a cat 3 days after operation.

C. Curves obtained from a dog 112 days after operation.

Fig. 10. Tonus curves of quadriceps muscles in cats.

A, *R*, tonus curve of the normal right quadriceps. *R'*, tonus curve of the same muscle after removal of the cerebellum.

B, *R* and *L*, tonus curves of right and left quadriceps muscles respectively following extirpation of the left lumbar sympathetic trunk. *R'*, tonus curve of the right quadriceps following removal of cerebellum. Tonus curve of left quadriceps, following removal of cerebellum, coincides with *L*.

further, in many of the experiments, after the tonus of both quadriceps was measured, in animals which had previously been subjected to unilateral extirpation of the lumbar sympathetic trunk, nicotin was administered in doses (1 to 2 cc. of a 0.5 per cent solution injected intramuscularly) which are generally regarded as sufficient to paralyze the sympathetic nervous system and the tonus of both quadriceps was measured again. In these experiments the tonus curves of both quadriceps obtained after the nicotin was administered were commonly identical and coincided with the tonus curve, obtained before nicotin was administered, of the quadriceps deprived

of its sympathetic innervation by operation. In some of these experiments nicotin was administered in such large doses (2.5 to 3 cc. of a 0.5 per cent solution) that the animals were kept alive only by means of artificial respiration. The tonus curves obtained for both quadriceps muscles, under these conditions, indicate no further reduction in tonus, but remain coincident with the curve obtained before the administration of nicotin for the muscle deprived of its sympathetic innervation by extirpation of the lumbar sympathetic trunk.

The results of the experiments outlined above show clearly that an important factor in the normal tonus of skeletal muscles is absent following elimination of their sympathetic nerve supply. This factor in the tonus of the quadriceps femoris muscles, which may be represented by the difference in the tonus curves obtained before and after sympathetic extirpation, includes that component of muscle tonus which is responsible for the brake phenomenon.

As is well known, the cerebellum plays an important rôle in the maintenance of postural tonus. In order to secure some measure of the cerebellar tonus and its possible relationship to the sympathetic nervous system, a series of experiments was carried out in which, after the tonus of both quadriceps muscles had been measured, in animals which had previously been subjected to unilateral extirpation of the sympathetic trunk, the cerebellum was removed and the tonus of both quadriceps muscles measured again.

The removal of the cerebellum is a somewhat drastic operation, but can be successfully carried out in the cat with little difficulty and without considerable loss of blood. In our experiments, great loss of blood was found to vitiate the results. In the operative procedure adopted, an incision was made along the dorsal midline from the tentorium to the level of the third cervical vertebra. The muscles were separated along the midline to the second cervical vertebra and detached from the occipital bone by blunt dissection. The bone was then broken away at the dorsal border of the foramen magnum to make an opening only as large as necessary. The meninges were divided with scissors. The cerebellar peduncles were cut, and the greater part of the cerebellar substance removed with a small blunt spatula. This operation, if successfully carried out, causes no apparent shock and is borne well by the experimental animal.

If, in a normal animal, the tonus of the quadriceps is measured, the cerebellum then removed and the tonus measured again either immediately or after an interval of several hours, it is found that the tonus has been materially reduced, though not to the same extent as by extirpation of the lumbar sympathetic trunk. The brake phenomenon, though less apparent than in the normal animal, is still present, as indicated by the slow rise at the beginning of the curve. The tonus curves of the quadriceps of the

same side obtained before and after removal of the cerebellum in a cat, are illustrated in figure 10, *A*.

If the tonus curves of both quadriceps are obtained, following removal of the cerebellum in a cat in which the tonus curves of these muscles have previously been obtained following unilateral extirpation of the lumbar sympathetic trunk, the curve obtained on the side on which the sympathetic nerve supply was eliminated commonly coincides with the curve obtained on this side before the cerebellum was removed, while the tonus curve of the muscle with its sympathetic nerve supply intact always falls between the curve of this muscle, obtained before removal of the cerebellum, and the coincident curves of the muscle on the opposite side, obtained before and after removal of the cerebellum (fig. 10, *B*). This experiment was carried out in ten animals with uniformly constant results. The reduction in the tonus of the muscle with its sympathetic nerve supply intact, due to removal of the cerebellum, was not equal in all cases, but was always unmistakable and never approached the reduction in tonus due to elimination of the sympathetic nerve supply. On the other hand, the tonus of a muscle deprived of its sympathetic nerve supply was in no case further reduced by removal of the cerebellum.

In a few experiments an attempt was made to destroy the vestibular nuclei in addition to removing the cerebellum. The results of these experiments do not differ materially from the results of the experiments outlined above. The tonus of the quadriceps with its sympathetic nerve supply intact still remained appreciably greater than that of the muscle without its sympathetic nerve supply. This muscle, however, suffered no further reduction in tonus due to removal of the cerebellum and destruction of the vestibular nuclei.

The results of these experiments indicate a reduction in muscle tonus following removal of the cerebellum which is less marked than the reduction in tonus following removal of its sympathetic nerve supply. Reduction in tonus following removal of the cerebellum is not greatly increased following destruction of the vestibular nuclei. Removal of the cerebellum and destruction of the vestibular nuclei are followed by no further reduction in the tonus of muscles deprived of their sympathetic nerve supply. These facts seem to indicate that the cerebellar and vestibular impulses which play a part in the brake phenomenon are conveyed to the skeletal muscles through the sympathetic nervous system. They also indicate that the sympathetic nerves convey to skeletal muscles additional impulses which are neither cerebellar nor vestibular in origin.

Discussion. The prevailing ideas regarding postural tonus in skeletal muscles have been derived largely from the studies of Sherrington and others on decerebrate animals. Sherrington (1896) observed that if, in a decerebrate preparation, the quadriceps femoris muscle were lengthened by

passively flexing the leg at the knee this position was retained. This he called the "lengthening reaction." If the same muscle were now shortened by passively extending the leg, the new position was again retained. This he called the "shortening reaction." According to Sherrington, the property of skeletal muscle which enables it to exhibit the "shortening" and "lengthening" reactions constitutes "plastic" tonus. He compared this property of skeletal muscle with that of involuntary muscle, e.g., the musculature of the urinary bladder, which enables it to adjust itself to an increasing or a decreasing volume without undergoing any material change in tension. Regarding this comparison he says: "Both are instances of postural contraction of muscle, though the relation of the central nervous system to the postural activity is very different in the two cases." Obviously, when this comparison was made, Sherrington assumed that the tonus of skeletal muscles is mediated solely through the somatic nerves.

Hunter (1924) and Royle (1924) vigorously advocated the theory that plastic tonus in skeletal muscles is mediated through their sympathetic, and contractile tonus through their somatic innervation. This is essentially the theory previously advanced by Langelaan (1915). However, Langelaan's papers do not show clearly that his conception of plastic tonus was quite the same as that of Sherrington. Hunter and Royle based their conception of plastic tonus directly on Sherrington's findings. They regarded it as a holding mechanism, i.e., that component of tonus which enables the muscle to take up a new length without appreciable change in tension. Their conclusion that plastic tonus is mediated through the sympathetic nervous system is based in part on Royle's findings in decerebration experiments following sympathetic extirpation.

As pointed out in the review of the literature, some of the studies of the effect of sympathetic extirpation on decerebrate rigidity have yielded only negative results. Others indicate reduction in, but not complete absence of decerebrate rigidity in limbs deprived of their sympathetic nerve supply. Recent investigators in this field have quite generally assumed that the theory advocated by Hunter and Royle implies two distinct kinds of muscle tonus each of which is mediated through a distinct system of peripheral nerve-fibers. Since decerebrate rigidity is a manifestation of exaggerated postural tonus, it has been argued that if plastic tonus is mediated through the sympathetic nerves, decerebrate rigidity ought to be not only reduced but absent in a limb deprived of its sympathetic innervation. This has given rise to a marked tendency to discredit the most important conclusions of Hunter and Royle.

Decerebrate rigidity is a tonic phenomenon in which the plastic and contractile components have not been clearly differentiated. Furthermore, Ranson's (1925) recent observation that decerebrate rigidity is reduced in the hind limb by application of a 0.04 per cent solution of

nicotin to the exposed dorsal root ganglia of the corresponding spinal nerves suggests that there is a nervous mechanism involved in the production of decerebrate rigidity which differs both from the ordinary somatic proprioceptive reflex arcs and from those which reach the muscles via the sympathetic rami. In view of this finding and the confusion which has resulted from studies of the effect of sympathetic extirpation on decerebrate rigidity, it seems quite probable that studies involving the phenomenon of decerebrate rigidity may not be well adapted to determine the exact rôle of the sympathetic nerves in the production of muscle tonus.

In a recent critical review Cobb (1925) maintains that it is "unproven and improbable that there is any qualitative difference between plastic tonus and contractile tonus." He regards muscle tonus as "a beautifully graded series of proprioceptive reflexes, continuously and unconsciously playing its part in our every motor act." He aptly points out that the most important afferent paths involved in the production of muscle tonus are those which arise in muscular and vestibular proprioceptive organs, and says, "the simplest reflex result of these afferents in the muscle is the myotatic reflex and from it may be built up by central nervous integration all the phenomena of postural contraction, excepting those which have their origin in the vestibular apparatus."

The experimental anatomical findings of Boeke (1913) and others which constitute the anatomical foundation of the theory of the sympathetic innervation of skeletal muscles can hardly be interpreted except as indicating that many proprioceptive reflex arcs reach the muscles through the sympathetic nervous system. The results of Hunter's (1924) experiment on birds, which are corroborated by the results of some of our experiments, also indicate clearly that when the somatic reflex arcs supplying the muscles of the wing are interrupted by cutting the dorsal roots of the brachial plexus, the proprioceptive reflex arcs which reach the wing muscles through the sympathetic rami still remain effective.

Apparently the sympathetic nerves include a goodly portion of the proprioceptive reflex system. There is no reason to assume that the quality of reflexes elicited by stimulation of proprioceptive receptors in the muscle is determined by the path of the peripheral components of the reflex arcs involved. However, the experimental date which indicate that the sympathetic system is a factor in the production of muscle tonus also indicate that the component of tonus which is mediated through the sympathetic system is essentially plastic in quality. But this is probably not the only factor in plastic tonus. The results of the present series of experiments indicate that the loss of the sympathetic component of muscle tonus in a mammalian limb, due to elimination of its sympathetic nerve supply, commonly results in no postural defect which may be detected by direct observation or palpation in the waking animal, although

appropriate methods of measurement reveal a marked reduction in tonus, in the absence of voluntary contraction. Neither do the tonus curves of the quadriceps femoris muscle, obtained after elimination of its sympathetic nerve supply, indicate complete absence of tonus in the resting muscle. The tonus which persists in this muscle, in the absence of contractile effort, is essentially postural and probably includes a component which is plastic in quality.

As observed in dogs under anesthesia, the reduction in tonus, following sympathetic extirpation, becomes apparent as soon as the voluntary nervous mechanism is in abeyance, and it is not completely compensated, following anesthesia, until voluntary control is quite completely restored. This is in full accord with Hunter's observation that the defect in the posture of the bird's wing, following section of the cervical sympathetic trunk, is increased under light anesthesia and by fatigue.

As pointed out by Spiegel (1923), the brake phenomenon is absent following interruption of the proprioceptive reflex arcs in pathological conditions involving degeneration of the dorsal root fibers, e.g., tabes dorsalis. It is, therefore, a reflex phenomenon. It does not differ in this respect from other elements of muscle tonus, since section of the dorsal nerve roots, if they contain both the somatic and visceral afferent fibers involved, is followed by complete atony of the muscles. On the other hand, section of the dorsal roots of nerves which contain only somatic fibers is not followed by complete muscular atony. As illustrated in the experiments on birds, postural tonus in the wing is not completely destroyed by section of the dorsal roots of the brachial plexus. A component of tonus is retained in the wing by reason of the fact that the afferent and efferent limbs of the reflex arcs involved are not components of the cervical, but of the upper thoracic nerves.

While muscle tonus is a reflex phenomenon, that component of tonus which is mediated through the sympathetic nervous system is not maintained by spinal reflexes alone. Comparison of the tonus curves of the quadriceps muscle with intact sympathetic nerve supply, obtained before and after removal of the cerebellum, shows clearly that the brake phenomenon depends in part on cerebellar reflexes. This was also pointed out by Spiegel. However, he maintained that the cerebellar impulses involved in the brake phenomenon are conveyed to the muscles through the somatic efferent fibers. Comparison of these tonus curves with those of the quadriceps deprived of its sympathetic nerve supply, obtained before and after removal of the cerebellum, shows further that cerebellar reflexes constitute an important factor in this component of tonus, and that those cerebellar impulses which play a part in the production of the brake phenomenon are conveyed to the muscles through the sympathetic nervous system. The results of those experiments in which the vestibular nuclei

also were destroyed indicate that impulses coming down from these centers likewise are conveyed to the muscles through the sympathetic nervous system.

Obviously the vestibular nuclei and the cerebellum are important reflex centers involved in the maintenance of tonus. Other prespinal centers involved are located in the midbrain. There is no evidence available which indicates that cerebral influences play an important part in the maintenance of postural tonus. Indeed, according to Magnus (1916), the tonus of skeletal muscles is not appreciably altered by removal of the cerebral hemispheres, or transection of the brain stem just below the thalamus. The importance of the mid-brain mechanism, in the maintenance of postural tonus, is suggested by the fact that transection of the brain stem at a somewhat lower level, viz., through the lower level of the red nuclei, releases the prespinal reflex arcs to produce decerebrate rigidity. Clinical observations seem to indicate that the corpus striatum also is a factor in the maintenance of muscle tonus. The present study affords no data bearing directly on the functions of the corpus striatum. It probably is a factor of considerable importance, at least under normal conditions.

A complete analysis of the central connections involved in the maintenance of muscle tonus is not within the scope of this paper. However, the experimental evidence at hand indicates that prespinal reflex centers play an important rôle in postural tonus, and that many of the descending fibers which subserve muscle tonus terminate in relation to the visceral efferent neurons in the intermediolateral cell column.

SUMMARY

Removal of the sympathetic nerve supply to a limb in dogs, by extirpation of the corresponding portion of the sympathetic trunk, usually produces no effect which can be detected by palpation or by direct observation during the normal behavior of the animal. However, when such animals are sujected to surgical anesthesia, the muscles of the limb deprived of its sympathetic innervation are more flaccid than those of the other limbs.

Extensor rigidity is usually less marked in the limb deprived of its sympathetic innervation than in the other limbs during the brief interval of excitation just before the anesthesia reaches the surgical stage.

Certain of Hunter's experiments on birds are repeated with corroborative results. Following section of the sympathetic trunk just caudal to the brachial plexus, the wing no longer fully maintains its characteristic flexed and adducted position while at rest. Following section of the dorsal roots of the brachial plexus, the muscles of the wing are not atonic, but tend to maintain the wing in any position in which it is passively placed. Following section both of the dorsal roots of the brachial plexus and of the cervical sympathetic trunk, the muscles of the wing become atonic.

A comparative study of the tonus curves of the quadriceps muscles, in cats and dogs, obtained before and after unilateral extirpation of the lumbar sympathetic trunk, and before and after removal of the cerebellum and destruction of the vestibular nuclei, indicates *1*, that the tonus of the muscle is very materially reduced and the brake phenomenon is absent following elimination of the sympathetic nerve supply; *2*, that cerebellar reflexes are an important factor in the component of muscle tonus which is mediated through the sympathetic nervous system; and *3*, that the tonus of the muscle deprived of its sympathetic nerve supply suffers no further reduction following administration of nicotin or removal of the cerebellum and destruction of the vestibular nuclei.

All manifestations of the influence of the sympathetic nervous system on skeletal muscles, except those of trophic character, are interpreted as manifestations of plastic tonus. Probably a component of plastic tonus may also be mediated through somatic proprioceptive reflex arcs.

The vestibular nuclei and the cerebellum are important reflex centers involved in the maintenance of postural tonus. Many descending fibers subserving postural tonus which carry impulses from these centers terminate in the visceral efferent column.

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BLOOD COUNTS IN VITAMIN-A DEFICIENCY DISEASE WITH ESPECIAL REFERENCE TO THE PLATELETS

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These experiments were undertaken in May, 1923, at the suggestion of Dr. Herbert Evans, Professor of Anatomy at the University of California. Our interest in this undertaking had been increased by the published report of Cramer, Drew and Mottram (1922). These investigators made counts on rats kept on a diet free from vitamin A and compared these counts with normal rats. Their counts included enumeration of the red cells and the blood platelets; attention being especially directed to the platelet enumeration. They state that their attention was drawn to the possibility of a diminution in the platelets of vitamin A deficiency rats because when the tail of these animals was cut for a blood count the blood flowed more freely and longer than normal. Their method of counting platelets was as follows:

> The animal is etherized and the tail placed in a watch glass containing a solution of 2 % sodium citrate in 0.6% NaCl solution. After cutting the tail and allowing the blood to flow until free bleeding is established the tail is transferred quickly to another watch glass containing about 1 cc. of sodium citrate, or Toisson's fluid. Blood is allowed to flow so that a mixture of the solution and blood convenient for counting red cells and the platelets is obtained. After the first few counts it is easy to recognize when the mixture is of convenient concentration. The tail is then wiped dry and the pipette of the hemocythometer is then filled with blood in the usual way so as to obtain a count of the absolute number of red cells. In order to count the platelets the citrate blood mixture is thoroughly stirred, a standard drop placed on a large slide and covered with a cover glass which is cemented with paraffine. After allowing the cells to settle the proportion of red cells to platelets in each field is counted until 30 platelets have been counted. From this the absolute number of platelets can be calculated.

Their counts on normal rats gave about 800,000 platelets per c.mm. of blood. The vitamin-A deficiency rats showed from 400,000 to 200,000 platelets per c.mm. They conclude that the platelet diminution in these rats varies with the duration and severity of the deficiency symptoms. The suggestion is brought forth that as the platelets decrease in number the resistance to infection decreases.

Our experiments were performed on rats from the experimental stock of Doctor Evans' Laboratory. These rats were all carefully pedigreed. They represent normals for control and animals in different stages of Vitamin A deficiency. The method employed by us for counting platelets was selected after considerable experience with several different methods. It seemed particularly applicable for use with such a small animal as a rat because it does not require more than a large drop of blood. The method is a modification of a method described by Anders Kristenson (1922). The solution used is as follows:

Urea	10 gm.
Sodium citrate	2.5 gm.
Corrosive sublimate	0.005 gm.
Brilliant cresyl blue	0.5 gm
Aqua distillatae	500 cc.

Centrifuge a small amount of this fluid for 10 minutes before using. This solution cannot be kept too long as spores develop in it which resemble platelets somewhat. We usually make up a new solution once each month. This solution is employed in the same manner as Toisson's or Gower's solution for counting red cells, except that in employing the red cell pipette the blood is drawn up to 0.1 instead of 0.05 and the pipette then filled with the above fluid. Shake well to thoroughly mix the contents of the pipette and to separate the platelets; then allow to stand for 10 minutes in order that the red cells will be laked and the platelets stained. The platelet estimation is made in exactly the same manner as the red cell estimation excepting that twice as much blood is used in the case of platelet counting.

In the beginning of our work with rats we tried to obtain our drop of blood from the sole of the foot after applying a moderately snug tourniquet and heat to the thigh. We soon gave this up as we encountered too much difficulty in getting a perfectly free flow of blood. This is, to our mind, the most important single factor in any method of platelet counting. If the drop does not come quickly and freely, or if tissue juices get mixed with the drop a grave error is introduced into the count at the outset. We next tried a method which worked out quite satisfactorily. The inside of the thigh was carefully shaved and cleansed with soap, alcohol, and ether. Then the internal saphenous vein was made to stand out by immersing the limb in warm water for about two minutes. With a sharp spicule of glass obtained by breaking a cover glass a quick firm stab was made into the vein and the first drop of blood drawn into the pipette as expeditiously as possible. If one wipes off the first drop and waits for the second there is more chance for tissue juices to affect the platelets and also for partial clumping of platelets to take place. The second drop was used for the red count and subsequent drops for the white count and for smears.

Complete counts were made in each animal by both authors separately and the counts checked against each other. Where there was a divergence of results the counts were repeated, if on repetition the counts still showed a considerable variation the count was not recorded. This only occurred at the beginning of our work and it was rather surprising how closely our counts tallied after we had gotten well into the work and became more familiar with handling and making counts on rats.

TABLE 1

Normal controls

ANIMAL NUMBER	WEIGHT	PLATELETS	RED CELLS	WHITE CELLS	P.M.N.	LYM.	P.M.N.E.	MONOS.	REMARKS
	grams								
1780	240	761,300	8,200,000	7,500					
1793	284	1,034,500	9,070,000	9,700					
1793	284	1,022,000	9,020,000	11,800					Counts made 3
1791	296	858,000	9,465,000	10,450					days apart
1784		1,064,000	9,540,000	16,700					
1782		800,000	9,500,000	11,000					
1797		872,000	9,200,000	9,600	26	62		12	
1791	304	1,188,000	9,210,000	8,800	27	54	5	14	
1795	296	1,140,000	9,135,000	12,600	23	46	18	13	Counts made 4
									days apart
1794	320	939,000	9,475,000	6,640	20	66		14	
1794		888,000	9,760,000	9,600	26.5	57	7.5	9	
1798	306	916,000	9,180,000	9,600	30	50	3	17	
1796	200	780,000	9,040,000	9,800	21	58	3	18	

The average count from the above table is:

Platelets	943,292
Red cells	9,215,000
White cells	10,291
Average P.M.N	24.8
Average Lym	56.0
Average P.M.N.E	7.3
Average Monos	14.0

A no. 15 Bausch and Lomb special compensating objective was used for platelet counting. Also, we found it necessary to use artificial light, either directly under the lens or just in front of the mirror. By the use of the above-mentioned diluting fluid the platelets are stained and also fixed so that they do not become fragmented and distorted and with a little experience it is easy to recognize and to count them with accuracy. The first counts were made on rats that were healthy and normal as far as could be ascertained. These counts may be tabulated as follows:

DISCUSSION. We see from table 1, counts made on eleven young,

TABLE 2

Vitamin-A deficiency animals

NUMBER OF ANIMAL	WEIGHT OF ANIMAL	PLATELET COUNT	RED BLOOD CELLS	WHITE CELLS	P.M.M.	LYM.	PMNE.	MONOS.	REMARKS
B 1513 1st count	189	730,000	9,510,000	18,600	29	50	8	13	Prolonged cornified beginning. No drop in weight. Eyes normal
B 1513 2nd count	150	704,000	9,360,000	11,500	28	62		10	This second count 3 weeks after the first. Animal on timothy 0.015 gm. daily. Steadily going down hill. No eye symptoms. Prolonged cornified.
B 1448 1st count	126	580,000	9,760,000	18,000	56	25	1	18	Prolonged cornified for 6 weeks. Xerophthalmia present. Marked loss of weight
B 1448 2nd count	118	536,000	9,290,000	16,500	67	17	3	16	Second count—4 days later—eyes are now destroyed
B 1448 3rd count	109	650,000	9,940,000	17,800	72	13	2	13	Animal in very poor condition. This third count 3 days after second count
W 1494 1st count	136	820,000	9,680,000	15,600	29	55	3	13	Xerophthalmia is questionable. No loss in weight. Prolonged cornified
W 1494 2nd count	160	750,000	9,800,000	9,400	26	64	3	7	Second count 2 weeks after first. On Turkestan alfalfa which is curative. Ga ning in weight. Cycles have been improved
B 1512 1st count	168	837,000	9,000,000	13,800	26	61	3	10	Interrupted prolonged cornified. Eyes normal. No loss in weight
B 1512 2nd count	168	760,000	8,690,000	17,000	33	53	4	10	Second count—4 days after first. Animal shows no change
W 1403 1st count	216	776,000	8,990,000	12,000	22	61	4	13	Interrupted prolonged cornified. No loss of weight. Eyes normal
W 1403 2nd count	218	700,000	9,300,000	13,200	17	69	2	13	No prolonged cornified. Eyes normal. Still gaining weight
W 1400 1st count	186	847,000	9,750,000	8,200	28	53	7	12	No prolonged cornified. No infection. Eyes normal
W 1400 2nd count	190	750,000	9,190,000	9,600	30	52	4	14	Going along normally. No prolonged cornified. No eye signs
	6 weeks later.								

W 1400	189	850,000	9,140,000	9,860	30	53	6	12	No change during 2 weeks' observation
3rd count	2 weeks later.								
W 1400	187	764,000	8,400,000	10,000	25	61	4	10	Prolonged cornified. Eyes show beginning xerophthalmia
4th count	3 weeks after 1st count.								
W 1227	120	610,000	9,880,000	14,600	55	28	4	13	Xerophthalmia. Prolonged cornified for 6 weeks. Marked loss in weight. Animal died 3 days after this count
W 1431	185	730,000	9,700,000	9,800	27	55	8	10	Eyes questionable. Prolonged cornified present. No loss in weight
1st count									
W 1431	185	764,000	9,470,000	12,560	23	62	2	13	No change in condition of animal
2nd count	1 week later.								
W 1276	92	732,000	9,780,000	12,000	32	52	6	10	Prolonged cornified for 6 weeks. Xerophthalmia present. Rapid loss of weight
1st count									
W 1276	81	824,000	8,600,000	14,600	76	15	5	4	Animal died on same day this count was made
2nd count									
W 1520	180	808,000	9,000,000	10,080	23	64	2	11	No loss in weight. Prolonged cornified for seven weeks. Xerophthalmia present. No lung infections
1st count									
W 1520	180	640,000	9,030,000	12,200	22	66	3	9	Prolonged cornified. Eyes show xerophthalmia
2nd count	1 week later.								
W 1520	150	776,000	9,080,000	6,400	67	19	4	10	Animal failing
3rd count	4 days later.								
B 1517	178	800,000	10,120,000	9,800	28	60	10	12	Prolonged cornified for one month—one interruption. No lung infection

Average weight	160	grams
Average number of platelets	739,083	
Average red blood cells	9,352,500	
Average white cells	12,629	
Average P.M.N.	36	
Average lym	48	
Average PMNE	4	
Average monos	11.5	

healthy animals yielded an average platelet count of 943,292; red cell count of 9,215,000; and white cell count of 10,291. It is interesting to contrast the counts made on animals in various stages of vitamin-A deficiency disease and to note the average platelet count in these animals of 739,083 which is 204,209 lower than the normal animal's average. The red cell count average is 9,353,500, an average 137,500 higher than the normal animals showed. This might possibly be due to alteration in blood volume as vitamin-A deficient animals seem to be dehydrated, giving us a concentrated blood. Some observations by Doctor Evans and his coworkers seem to bear this out. Such concentration would alter the red cell count more than the other elements of the blood. A comparison between the white cell counts of the normal and the deficiency animals shows a slight rise in the latter animals with an inversion of the polymorphonuclear-lymphocytic ratio. This is to be expected from the fact that so many of the deficiency animals have low grade infections.

Thus results with respect to the platelet counts in Vitamin A deficiency animals do not agree with those of Cramer, Drew and Mottram and we do not feel that the assumption has been proven that a decrease of platelets to a certain level—say 300,000— is the direct determining factor for loss of immunity and invasion of bacteria leading to such lesions as xerophthalmia and broncho-pneumonia.

Such a theory is extremely attractive but lacks, at present, sufficient proof for its unqualified acceptance. The published results of platelet estimations in vitamin-A deficiency rats of S. Phillips Bedson and S. S. Zilva (1923) are in agreement with our results. The greatest diminution which they found in deficiency animals was 244,000, or 21.3 per cent.

CONCLUSIONS

1. Twenty-four blood counts made on rats in various steps of vitamin-A deficiency showed an average number of platelets 204,209 lower than our normal animals; an average number of red cells 137,500 higher than the controls; and an average number of white cells 2338 higher than the normal control with an inversion of the polymorphonuclear-lymphocytic ratio.

2. From these results we conclude that changes in the relative number of platelets, red cells and white cells in the blood of rats are not striking enough, or constant enough, to constitute specific lesion of vitamin-A deficiency in these animals.

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SUPRARENAL TRANSPLANTATION IN THE DOG[1]

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The possibility of the transplantation of the suprarenal glands being used as a therapeutic measure in Addison's disease has led to a great deal of work on the dog, cat, rabbit, rat and guinea pig Many different sites have been used for the location of the graft, e.g., kidney, thyroid, testis, omentum, muscle and subcutaneous tissues. There are also on record a few cases in which the suprarenal gland of an animal has been transplanted into a human being suffering with Addison's disease. Thus, Cohoe (1922) cites Bra as having transplanted the suprarenals of a dog into the cellular tissue of the abdomen in the case of a child with Addison's disease, but the operation was followed by death in three days. Jaboulay (1897) reported two cases of Addison's disease with death within twenty-four hours following suprarenal transplantation.

The results of suprarenal transplantation in animals are quite conflicting, probably because several factors with important bearings on the interpretation of the results have not been considered. Some investigators have apparently failed to take into account the frequent occurrence of accessory suprarenal glands or tissue in the animals they used. Others, working under the impression that it is the medullary portion of the suprarenal gland which is necessary for life, have carefully shaved off all the cortex of the gland before transplantation. In some cases the operative technique employed and the methods of transplantation used have been such as to make questionable the conclusions reached. Thus von Haberer (1908) reports successful transplantation of the suprarenal gland in 50 per cent of his cases using the kidney as the site of the graft. However, in studying his method, it is found that he transplanted the gland into the kidney while it was still partly attached by a pedicle to its original location, so that a portion of the original blood supply of the gland was left undisturbed, and there was continuity of tissue between the extrarenal and intrarenal portions of the gland. This is not true transplantation and its practical importance is limited.

[1] The data here presented are taken from the thesis submitted to the Faculty of the Yale University School of Medicine in candidacy for the degree of Doctor of Medicine, by Israel Blodinger and Harry E. Klebanoff.

Canalis (1887) was one of the first to attempt suprarenal transplantation in animals. He grafted small portions of the gland into the kidney, but found that in all but one case the grafts became necrotic and were absorbed. Abelous (1892), Boinet (1895), and Busch and Wright (1900) have reported unsuccessful results with grafts in various parts of the body. Stilling (1915) after transplanting suprarenals into the testes was able to find cortical tissue as late as three years following operation. Coenen (1906) reported negative results. Whether implanted whole or in pieces, the transplanted suprarenals were always absorbed at the end of two months and nothing was left but a small mass of pigment in granulating cells.

Busch and vonBergen (1905) believed that the lack of success attending transplantation or grafting of the suprarenals was due to the difficulty of preservation of the medulla which they state alone contains the active principle and which is functionally essential. Everyone now feels it is the cortex which is essential. Busch, Leonard and Wright (1908) published the results of 30 cases of transplantations into the thyroids, testes, and kidneys of dogs and rabbits, not only in the same animal but also from one animal to another of the same species, and conclude that there is satisfactory evidence of functionating suprarenal graft survival in at least three cases. In several other instances where complete proof could not be obtained, they felt that there was a strong probability that the grafts were functionating. Stewart (1924) gives some data on the length of life after double adrenalectomy in dogs when the operation is done in one or two stages.

With the above situation in view it seemed of interest to attempt to find out whether or not it is possible to obtain physiologically functionating transplants in dogs. The dog was chosen because of the relative infrequent occurrence of accessory suprarenal glands or tissue. With one exception autotransplantation was done with the thought that if it should prove successful homotransplantation and heterotransplantation would be attempted. The most modern aseptic methods were observed throughout. Every attempt was made to develop an adequate surgical technique and since none of the animals died after the first operation,—that is, of removing one adrenal and transplanting the whole or a part of it,—and since the immediate reaction of all animals was good to both operations we feel that this was the case.

The outline of procedure was as follows: A first operation was performed in which the left suprarenal gland was entirely removed, and the whole or a part of it transplanted into the kidney, spleen or other tissue chosen as the site. After a varying interval, a second operation was performed, at which the entire right suprarenal gland was removed. Then, should the animal survive a sufficiently long period of time, a third opera-

tion was to be done, which would consist essentially in removal of the transplanted suprarenal tissue. If this operation were followed by death within 1 to 3 days, preceded by marked muscular weakness and tremor on exertion, then it would be probable that suprarenal insufficiency was the cause of death and that the graft had been functioning, provided autopsy revealed no accessory suprarenal tissue and histological examination showed that the graft was alive when removed. Following is a description of the technique employed.

First operation. Morphine and atropine pre-operatively. Ether anesthesia. The abdomen is shaved and prepared with iodine and alcohol. An incision is made on the left side, just within the mammary line, from the costal margin extending posteriorly 3 to 4 inches. The left suprarenal gland is exposed and dissected out in its entirety, with little or no hemorrhage. The edges of the gland are freshened, preserving as much of the cortical substance as possible. The whole gland, or a sagittal portion of it, is then introduced into the organ chosen for the site of transplantation, into an opening patterned as far as possible after the shape of the graft to be transplanted and held in place by two silk sutures. The graft is, in every case, completely buried in the tissue wherein transplanted and care is taken to prevent the intervention of blood clot between the surfaces of the graft and of the tissue receiving it. The abdomen is then closed in layers and a dry dressing applied to the wound.

Second operation. Using the same general method as above, the right suprarenal gland is removed in its entirety. Careful dissection is necessary due to the close relation of the right suprarenal gland to the vena cava.

The following typical histories, including autopsy findings, will illustrate the results.

No. 1. 11–27–23. Left adrenalectomy. The animal stood the operation well and in 24 hours was in good condition. Convalesced rapidly and remained in excellent general condition.

1–17–24. Right adrenalectomy (51 days after first operation). Immediate post-operative reaction good. Three hours after operation the animal made efforts to stand, but was very weak. Soon became apathetic and no longer responded to stimulation and died 8 hours after operation. Autopsy revealed no hemorrhage nor any other obvious cause of death.

No. 4. 12–14–23. Left adrenalectomy, half of gland transplanted into the cortex of the left kidney, other half into the spleen. The animal appeared in good condition several hours after operation and remained in excellent general condition.

1–8–24. Right adrenalectomy (25 days after first operation). The animal appeared quite well after operation. Six hours after operation, able to stand up and drank some water. Later would only stand on forelegs, hind legs apparently unable to support weight of body. Finally no effort at all to stand and a slight tremor noticeable in forelegs. Became more apathetic, no longer reacting to stimulation and died 18 hours after operation.

Autopsy. No evidence of infection or hemorrhage. Adhesions of the omentum

to the left kidney and to the spleen. Small linear scars on the surface of the kidney and of the spleen marking the points of transplantation. Cross-section of the kidney showed a small whitish area about half the size of the original graft. On cross-section of the spleen a reddish white mass about ¼ the size of the original graft was seen, surrounded by apparently normal splenic tissue.

Microscopic section of the kidney graft showed it to be surrounded by apparently normal kidney tissue. No definite cortical or medullary cells could be seen. In this area were some broken down cells whose exact nature could not be determined. At the outer edges of this area were many fibroblasts and plasma cells, but no polymorphonuclears.

Microscopic section of the spleen showed a structureless area with a few mononuclears surrounded by normal splenic tissue. No evidence of infection.

No. 6. 12–19–23. Left adrenalectomy, half of gland transplanted into the cortex of the left kidney, other half into the spleen. Animal recovered rapidly and remained in excellent general condition.

1–22–24. Right adrenalectomy (34 days after first operation). Normal post-operative reaction. Twelve hours after the operation the animal was drinking water freely but ate no food. Able to stand for only short periods of time. Twenty-four hours after the operation took milk powder (Klim) and water. Able to stand on all four legs for periods of 5 to 10 minutes, after which hind legs appeared to give way and would lie down. Drank water freely. Forty-eight hours after operation the animal stood only when forced to do so and would slowly lie down again. Took a little Klim mixture. Apparently weaker and 72 hours after operation made no effort to stand even when forced. Refused Klim mixture, but took a little water occasionally. Finally made no attempt to move even when stimulated and a slight tremor of head and legs became apparent. Ninety-four hours after operation the animal died.

Autopsy. A careful search revealed no evidence of accessory suprarenal tissue. No peritonitis nor evidence of any other infection. Strong vascular omental adhesions over the left kidney, and to a lesser extent over the spleen. Small depressed scars on the surfaces of the spleen and left kidney marked the points of transplantation.

Cross section of the left kidney showed a whitish area about half the size of the original graft, closely surrounded by apparently normal kidney tissue. On cross section of the spleen a yellowish-white soft area was seen about ⅓ the size of the original transplant.

Microscopic section of the area in the left kidney showed it to be surrounded by apparently normal kidney tissue. Beneath this was a layer of dense young connective tissue, with many fibroblasts and many new engorged blood vessels. Within this layer was a heterogeneous area consisting of groups of apparently cortical cells. Most of them had lost their nuclei and many were undergoing necrosis while a few were apparently normal. Interspersed between these clumps of cells were many phagocytes, chiefly plasma cells. Throughout the entire central portion of the area was much finely granular pigment. No definite medullary cells could be made out.

Microscopic section of the splenic area showed normal splenic tissue surrounding an area made up of a few cords of degenerating cells, probably cortical. There were many phagocytic cells, some red blood cells and a great amount of pigment. No medullary cells could be seen.

No. 9. 1–10–24. Left adrenalectomy, half of gland transplanted into the cortex of the left kidney, other half into the omentum. The animal made complete recovery and 5 days after was in excellent general condition.

1–15–24. Right adrenalectomy (5 days after first operation). Immediate post-

operative reaction good. Eight hours after operation the animal was able to stand on all four legs for short intervals of time, but would quickly lie down as hind legs seemed to give way. On opening cage, would stand up and soon lie down again. Twelve hours after operation no longer made efforts to stand when cage was opened, but would feebly raise head when touched, the movements being accompanied by definite tremor. The animal refused all food and drink and died 16 hours after operation.

Autopsy. No evidence of infection or hemorrhage. There was a slight omental adhesion over the left kidney which was easily separated. The surface of the kidney showed the usual scar.

The region of the omental transplant showed a hard, much vascularized nodule of omental tissue, in the middle of which was the suprarenal graft, practically the same size as the original, but with the cortical and medullary tissue less clearly defined.

Microscopic section of the omental graft showed from without inward: A layer of normal omental tissue.

A layer of rather dense connective tissue containing a large number of small and large blood vessels. There was some mononuclear infiltration in this layer. No polymorphonuclears were present.

A layer of apparently normal cortical tissue, but which on closer examination showed many of the cells to have lost their nuclei, many with pyknotic nuclei, while some were entirely disintegrated. No cellular infiltration in this layer.

The center of the area which should have been the medullary part of the gland showed an indefinite, structureless area with here and there a trace of groups of cells and scattered pigment.

Microscopic section of the kidney graft showed it to be separated from apparently normal kidney tissue by a very thin band of connective tissue, under which there were cortical cells in various stages of degeneration. There was a small amount of mononuclear infiltration, no polymorphs. In the medullary portion were many structureless areas between which were interspersed groups of cells, of which only a few appeared normal.

No. 11. 1–2–24. Left adrenalectomy, about three-quarters of gland taken immediately from another dog under anesthesia was transplanted into the cortex of the left kidney. Both dogs were mongrels. The immediate post-operative reaction was good. In a short time the animal was entirely recovered and in excellent general condition.

2–6–24. Right adrenalectomy (25 days after first operation). Immediate post-operative reaction normal. Six hours after operation stood for short periods of time when forced to do so. Twenty-four hours after operation less active, walking around room when taken out of cage, but running only when forced. Seventy hours after operation appearance strikingly different, lay in cage and stood up only when moved and then immediately lay down again. On being taken out of the cage, stood quietly for a few moments and then lay down, seeming reluctant to move. Drank a little water, but refused Klim mixture, of which he had eaten quite a bit during the previous 48 hours. Finally no longer any effort to stand up even when forced. Lay on side, breathing quietly and only occasionally feebly raising head when touched. Remained in this condition until death, 95 hours after operation.

Autopsy. No evidence of infection. A careful search revealed no accessory suprarenal tissue. The usual adhesions of omentum to kidney were present. A linear scar was seen at the surface of the kidney at the point of transplantation. Cross section of the graft showed a small mass about ⅓ the size of the original graft, yellowish-white in color, separated from apparently normal kidney tissue by a very narrow whitish ring.

Microscopic section showed normal kidney cortex surrounding an area showing on the outside a band of dense young connective tissue with many fibroblasts and young blood vessels. Within this layer could be seen clumps of necrotic cells, which appeared like cortical cells, with an infiltration of phagocytes, mostly plasma cells. In the very center of the area was a structureless mass containing an abundance of pigment.

Our attempt to obtain functionating autogenous grafts of the suprarenal gland in the dog was thus not successful. Speculation as to the reason will not be carried beyond the statement of an obvious view, namely, that the gland is too highly specialized to take when transplanted and that lack of an intact sympathetic innervation may have something to do with this. If these results are confirmed with regard to autogenous transplantation, homogenous and heterogenous transplantation seem hopeless.

TABLE 1

Time interval between first and second operation and number of hours animal survived second operation

	DOG 9	DOG 12	DOG 5	DOG 8	DOG 15	DOG 4	DOG 11	DOG 13	DOG 3	DOG 7	DOG 6	DOG 2	DOG 10	DOG 16	DOG 14
Time in days between first and second operation	5	14	19	22	24	25	25	25	30	30	34	41	41	74	80
Survived second operation, hours	16	20	24	18	16	18	95	18	18	12	94	15	20	19	15

We are at a loss to explain why the dogs, or some of them, did not live longer. Stewart and Rogoff (1925) found after double adrenalectomy, with an unstated interval between the removal of the two glands, that dogs would remain alive as long as 16 days though most of them die within a week. We are, of course, convinced that the early death of our dogs was not due to lack of operative care and believe that it is specific to the removal of the glands. All the animals showed the characteristic weakness of the hind legs, tremor, and loss of appetite. The fact that a graft was made in addition to the removal of both glands could hardly have added anything in the way of insult to the animal.

SUMMARY

Suprarenal transplantation was performed in 15 dogs. In 14 the animal's own gland was transplanted into another portion of the body (autotransplantation), and in 1 case a suprarenal gland taken from another dog was transplanted (homotransplantation). The sites chosen to receive the graft were the kidney, spleen, omentum and muscle (rectus).

Second operations, consisting in the removal of the non-transplanted gland, were done at intervals ranging from 5 to 80 days after the first

operation. Following the second operation, death occurred in every case with symptoms of suprarenal insufficiency. In 13 cases death resulted in less than 24 hours after operation, the average being 18 hours. The remaining two animals lived 94 and 95 hours respectively after the second operation, succumbing with typical symptoms. In one animal (no. 1) a two-stage double adrenalectomy with no transplantation was done, the dog dying 8 hours following removal of the second gland.

General autopsy findings were as follows: No evidence of infection. Early acquisition of blood supply by the transplant microscopically demonstrated in all cases by extensive vascular reaction in the tissue immediately surrounding the graft. Apparently living (cortical) cells seen in some cases as late as 34 days after transplantation. Apparently living medullary cells seen in but one case, a graft 5 days old. No suprarenal cells seen later than 34 days following transplantation, the graft being then completely destroyed and partly or completely replaced by connective tissue, phagocytic cells and some pigment.

The kidney seemed to be the best site for transplantation, the graft apparently being kept alive longer than in other sites used. The grafts in muscle were most rapidly destroyed and absorbed.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE GASTRIC SECRETION

X. FORMATION OF HYDROCHLORIC ACID BY THE GASTRIC MUCOSA

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In spite of the large amount of time devoted to the study of the site and manner of production of hydrochloric acid through the activity of the gastric mucosa, there are still two opposing views regarding this question. The studies of Miss Fitzgerald (1910), Hammett (1915), and Collip (1920) tend to show that the hydrochloric acid is formed, at least in part, within the parietal cell. Harvey and Bensley (1912), on the other hand, find that while the parietal cells may form the precursors of the acid, they do not produce the acid itself, the secretion formed by these cells being, according to them, mildly alkaline in reaction.

Good reviews of the earlier work on this subject may be found in the articles by Fitzgerald, Harvey and Bensley, and Collip. Accordingly, we will confine our discussion of the literature to the more recent papers which have a direct bearing on the problem.

Fitzgerald (1910), working with rabbits, dogs, and guinea pigs, employed the Prussian blue reaction to reveal the site of the origin of the hydrochloric acid found in the gastric juice. She used weak neutral solutions of potassium ferrocyanide and the citrate of iron and ammonia, and concluded that the occurrence of the Prussian blue reaction in the canaliculi of the parietal cells, following the introduction of these solutions into the circulation, afforded conclusive evidence of the presence of free acid in these structures. She believed that the acid was formed free in the cytoplasm of the parietal cell itself, from which it diffused into the canaliculi.

Harvey and Bensley (1912) repeated Fitzgerald's work but used much more concentrated solutions of the iron salts. They also introduced new methods of study. From their work with iron salts they concluded that Fitzgerald's conclusions were unjustified. By making use of dyes, chiefly cyanamin bichloride and neutral red, which give different colors in alkaline and acid media, Harvey and Bensley found that free acid was present

only on the internal surface of the stomach and in the foveolae. The canaliculi of the parietal cells gave an alkaline reaction. They advanced the hypothesis that the parietal cells secrete a chloride of an organic base, this compound yielding free hydrochloric acid only as it reaches the gastric foveolae.

Hammett (1915) critically reviewed the evidence put forward by both Fitzgerald, and Harvey and Bensley, in regard to the site of hydrochloric acid formation. He concluded that the reasons which led Harvey and Bensley to reject the results obtained by Fitzgerald, using the Prussian blue reaction, were inadequate. In regard to the results obtained by the immersion of excised portions of the mucosa in cyanamin and neutral red, Hammett pointed out that the tissue after removal from the organ does not continue its secretory function. The results observed, he maintained, consequently would be dependent on the relative velocities of diffusion of the dye and the acid. Since the dye diffuses less rapidly than the acid, Hammett held that the results observed by Harvey and Bensley were to be expected, and that the reaction in the canaliculi and lumen of the gland would be alkaline following the removal of the acid by diffusion.

Collip (1920), in an extended comparative study of acid formation in several classes of vertebrates, confirms Miss Fitzgerald's findings and accepts her interpretation of them. He believes that the hydrohloric acid cannot occur in any quantity in the parietal cells themselves, but diffuses quickly, as it is formed, into the intracellular canaliculi. By microchemical tests he was able to show that phosphates, and very probably carbonates, were abundant in the cytoplasm of the parietal cells of the resting mammalian stomach, while chlorides were absent or occurred only in traces. Chlorides were not taken up by the parietal cells until they were stimulated to secrete. Collip also found that chlorides might be absent from the parietal cells at points or restricted zones in the actively secreting stomach, indicating that not all the gastric glands or parietal cells are in full secretory activity at any one time. This distribution of chlorides, phosphates, and carbonates in the gastric mucosa in different stages of activity justifies, according to Collip, the adoption of the Maly theory (1878) as to the mode of origin of the hydrochloric acid of the gastric juice.

The methods employed by the investigators referred to above were not strictly physiological. Dyes were used whose action on gastric secretion was not known and not determined. The animals were rendered unconscious in order to obtain gastric mucosa for study by various methods, which, we know, abolish, or are incompatible with gastric secretion; and in some instances (injection of dye into the coeliac artery), the blood flow to the stomach was interfered with. For this reason we have felt for some time that much physiological importance could not be attached to the observations that have been made.

In this paper we will report the results of a partial repetition of the work of the above investigators, using a method that obviates for the most part the above unphysiological conditions.

METHODS. In our work we have used Pawlow pouch dogs, the pouch being made with a large opening so that pieces of mucosa from the mid-portion of the pouch could be obtained for histological study. With such a preparation we could follow the elimination of the dye, and its effect on gastric secretion; and obtain pieces of mucosa from an actively secreting stomach and make a histological examination within a minimum period of time (usually two minutes). See figure 1.

The only unphysiological aspect of our method is that the piece of mucosa studied was deprived of its blood supply for a period of about two minutes prior to direct observation. Hence we believe that this method can only be surpassed by the possible method of direct visualization in situ of the gastric glands, which we have not yet been able to accomplish.

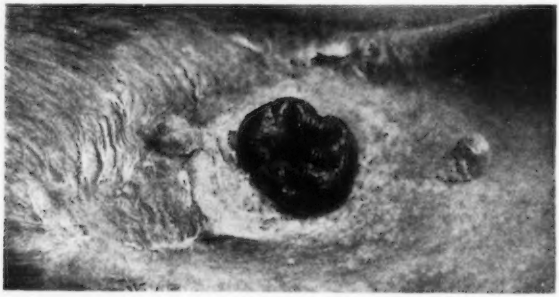

Fig. 1. This photograph shows the rosette of a Pawlow pouch so prepared that small pieces of mucosa could be excised from the mid-portion of the pouch.

We examined the mucosa both after intravenous administration of the dye and after immersion of a piece of mucosa in a suitable solution of the dye.

EXPERIMENTS AND RESULTS. *The elimination of dyes and their effect on gastric secretion.* We first determined the dyes that were eliminated by the gastric mucosa, noting their toxicity, their effect on gastric secretion, the dosage required, the time of appearance, and the time of maximum concentration in the gastric juice. These results have been published (Dawson and Ivy, 1925). Some of the dyes could not be used because of their toxicity, others because they were not adequate indicators of pH, and others because they were eliminated in an insufficient concentration to give the parietal cell a definite color.

As a result, most of our experiments involved the use of cyanamin bichloride, neutral red, and iron salts.

Results using cyanamin bichloride.[1] *1. Intravenous injection.* Cyanamin bichloride is only slightly soluble in normal saline and when it is introduced intravenously in a saturated solution (50 to 150 cc.), it is eliminated by the gastric mucosa of a normally secreting Pawlow pouch dog only in traces (Dawson and Ivy, 1925). The gastric juice is sufficiently blue in color to definitely recognize the presence of the dye. When the mucosa is examined, however, at various intervals before and during the appearance of the dye in the gastric juice, we find it impossible to definitely distinguish the dye in any of the cells of the mucosa. This dye, then, is eliminated by the physiological gastric mucosa, but in too dilute concentration to render it visible.

2. Immersion of excised portions of actively secreting gastric mucosa in cyanamin bichloride. Pieces of mucosa from the actively secreting pouch were immersed in a saturated solution of the dye according to the method of Harvey and Bensley. The color reactions obtained by them were also obtained by us. At this point, the pH was determined[2] at which the cyanamin bichloride that we were using gave a definite blue tint. The result was a pH of 3.0.

Many writers in quoting Harvey and Bensley have apparently overlooked this point, although these investigators clearly pointed out the fact that cyanamin was not a delicate indicator.

The result then, obtained by them and by us with this dye, only indicates that at the moment of their and our observations, the cytoplasm of the parietal cell had a pH greater than 3.0.

Results using neutral red. Neutral red is a very suitable dye for physiological studies on the stomach. It has a "working range" of pH 6.8 to 8.0 (being crimson at pH 6.8 and yellow at 8.0), is not toxic even in large quantities at a high concentration, and is eliminated freely and rapidly by the fundic mucosa following intravenous injection (Dawson and Ivy, 1925).

1. Direct application of neutral red to the fundic mucosa. We first attempted to stain the mucosa directly by filling the actively secreting pouch with the stain, a solution of 1–2,000 being used, and retaining it in the pouch for periods varying from fifteen minutes to two and one-half hours. After application of the dye small pieces of the mucosa were clipped and examined microscopically for staining. The stain was found to be limited to the surface and foveolae, and apparently had not passed into the lumina of the gland tubules.

Following these observations, efforts were made to apply the dye under

[1] Prepared through the kindness of Dr. W. C. Austin, Department of Bio-Chemistry, Loyola University Medical School.

[2] This was determined for us by Dr. W. C. Austin with buffer solutions and checked electrometrically.

pressure, using an applicator similar to the one described by Ivy and Whitlow (1921). Although the dye was kept in the pouch under a continuous pressure for varying periods of time, no staining further than that noted above, was obtained by this method.

2. Intravenous injection of neutral red. Intravenous injections of a saturated solution of neutral red in normal saline were employed. Ordinarily 50 cc. were injected. The gastric juice, following such an injection, took on a very distinct crimson color in from three to five minutes, but neither the quantity nor quality of the secretion was changed.

At various intervals after the injection, but usually at the height of elimination, pieces of the mucosa were clipped from the pouch, rinsed in normal saline and placed on a glass slide with the cut surface down so that the secreting surface could be observed under the low power of the microscope. By this method it was found that not all of the foveolae contained brightly stained secretion, but that the crimson secretion masses were scattered irregularly over the surface in groups, usually five to eight in a group.

This selective elimination of neutral red indicated that not all of the gastric glands are in full activity at one time, confirming Collip's conclusion arrived at from entirely different evidence.

In other experiments, pieces of mucosa were clipped, rinsed and placed on the slide with the free surface down. The tissue was then quickly teased with needles, the motion being from the center to the periphery, and the time elapsing from the time of clipping to the time of observation never being over three minutes. By the teasing, some of the tubular glands were dislodged from their position and dragged out so that their fundi projected beyond the edge of the excised tissue. It sometimes occurred out of many trials, because we were working against time, that a gland whose crypts contained a crimson secretion mass was dislodged and accordingly could be examined under the high power of the microscope. We observed that the staining of the parietal cells was diffuse, but the delicate tint was crimson, indicating that under the conditions of the experiment the parietal cells of these active tubules were at least slightly acid in their reaction.[3] On account of the diffuse nature of the staining no trustworthy observations on the location of the dye in the cells could be made. However, it appeared to be in the granules. There was no storage of the dye in the granules, as it was apparently eliminated from the cells almost as fast as it entered. The most intense staining occurred in the crypt. The crimson color of the lumen was less conspicuous.

3. The immersion of clipped pieces of mucosa in neutral red. In this work we followed largely the method employed by Harvey and Bensley. The two chief differences were: first, we obtained our material directly

[3] This was demonstrated several times to Doctors Luckhardt and Carlson.

from the actively secreting mucosa (Pawlow pouch) of living dogs, while the animals used by them had either been bled to death or stunned and the gastric mucosa then exposed by opening the abdominal cavity and cutting away the muscular layers of the stomach; second, after having rinsed the excised tissue in normal saline, it was teased in the dilute solution of neutral red, instead of being first teased and later immersed in the stain. Our efforts in this experiment were directed toward eliminating as far as possible any disturbance to the secretory mechanism, and to shortening the period of time elapsing between the disturbance of the mucosa and the moment of microscopic observation. Our earlier observations on the direct application of neutral red showed that clipping a portion from the mucosa had a profound effect on the tissue excised. Presumably the secretory process is immediately interrupted by such a radical procedure. The mucosa from some pouches resisted teasing while that from others teased readily. The differences in teasing apparently can be accounted for by a difference in the length of time since the pouch was made. Pouches of long standing appeared to have an increase in fibrous tissue which made teasing difficult.

In the preparations made in the manner just described, the staining of the crypts and the small granular cells of Heidenhain was as described by Harvey and Bensley, but the lumen of the fundus of the gland and the canaliculi of the parietal cells also showed the acid reaction at the moment of staining. The crimson color, however, was soon displaced by the orange-red of the neutral reaction and quickly passed over to the yellow of the alkaline, the time interval usually being not longer than two minutes. The variability was due probably to the different amounts of time consumed in the teasing process. This progressive shift to the alkaline side was often emphasized by the contrasting behavior of the spirilla which are usually found in the canalicular apparatus. These organisms take a heavy red stain which they retain, and as the color reaction in the canaliculi changes to yellow these minute forms become more and more conspicuous.

In one preparation the expanded end of the ductule which the canaliculi enter was observed to undergo changes in size, becoming enlarged and then decreasing in size. This was apparently not due to pressure from the cover slip. Another feature of the staining by the immersion method should be noted. It often occurs that some tubules which are as well isolated by teasing and in as advantageous a position as others, fail to take the stain. This must be regarded as another evidence of regional activity in the secreting stomach. It has been observed by Harvey and Bensley that the resting tubules do not take up cyanamin.

The evanescent crimson color stands in great contrast to the yellow which replaces it, as the alkaline tint persists for a long time. Preparations left standing as long as twenty minutes show the staining picture

described by Harvey and Bensley—yellow canaliculi with a gradually increasing red tint until the crimson of the crypt is attained. Preparations made from pouches in which the secretion is known to have been abolished by toxic materials, also present the color gradations just described, but do not show the primary crimson tint referred to above.

In summarizing our observations with neutral red, we can state that we have observed three stages of staining in the parietal cells of the actively secreting mucosa. A primary stage, in which the cytoplasm, or canaliculi, depending on the method used, present a crimson color indicating a pH on the acid side of neutrality. A secondary stage, occurring in a few minutes (3 or 4 minutes) after removal of the piece of mucosa or cessation of secretion, in which the cytoplasm, or canaliculi, depending on the method used, present a yellow color indicating a pH on the alkaline side of neutrality. A tertiary stage, occurring after a period of fifteen or twenty minutes following removal of the specimen, in which there is a reversal back to the red and crimson, or to acid reaction. By the immersion method, the lumen of the fundus and the canaliculi stain crimson or acid; but by the intravenous method, the cytoplasm stains diffusely crimson or acid. Our observations differ from those of Harvey and Bensley only in that we observe a primary stage in which the parietal cell stains acid to neutral red.

Results using iron salts. In the work with iron salts we used the mixtures of Fitzgerald (1910) and of Collip (1920). Fitzgerald's solution is prepared by mixing equal parts of 2.25 per cent solution of ammonium ferric citrate and 1.5 per cent solution of potassium ferrocyanide. Collip's solution is made by adding 10 parts by volume of a 3 per cent solution of sodium ferrocyanide to 7 parts of a 4 per cent solution of ammonium ferric citrate.

In large quantities, the iron salts are markedly toxic whether introduced subcutaneously or intravenously. Usually the iron solution was introduced in small quantities, 15 to 25 cc., at intervals of from 15 to 20 minutes, the subcutaneous and intravenous routes being utilized alternately. By proceeding in this manner, it was possible to note the tolerance of the individual dog and to stop injecting before the animal became sufficiently intoxicated to cause a depression of the secretory activity. In overdoses, the mucosa of the pouch blanched and exhibited marked motility. This was often followed by vomiting and cessation of secretion, which sometimes was continued for quite a long period. Gastric secretion would sometimes be completely inhibited even though vomiting did not occur. Fitzgerald's mixture proved much more toxic than that of Collip. This was probably due to the presence of potassium in the former, as Collip's mixture contains sodium ferrocyanide.

With a single dose of iron salts, the Prussian blue reaction is not obtained

on the free surface of the mucosa. After two to three injections are made in the manner described, small blue patches can be seen in the mucus with the naked eye. There is, however, no general elimination over the entire surface, the blue patches being limited to small areas. When observed under the low power of the microscope, the blue patches are found to be made up of groups of small blue masses which lie in the crypts, but all crypts do not contain the precipitate.

In one animal we obtained results very similar to those described by Fitzgerald. The iron was precipitated in the canaliculi and the parietal cells themselves had a blue-green tint. In this dog, however, secretion was inhibited and finally ceased entirely. This was followed by violent vomiting. The animal was greatly depressed following the experiment, and refused food for two days.

With Collip's mixture, on the other hand, we were unable to obtain these results. The Prussian blue reaction was obtained on the free surface as before, but iron could not be detected in the parietal cells or in the lumina of the glands. It was limited to the crypts. Portions of the mucosa showing the precipitate were clipped and fixed in both alcohol, and in formalin containing hydrochloric acid. When sectioned, neither tissue showed the iron in any position other than the mouths of the crypts.

Effects of concussion on gastric secretion. Since some workers obtained their material from animals rendered unconscious by a blow on the head, it seemed advisable to ascertain the effect of this procedure on secretory activity. For this experiment a Pawlow-pouch dog was used. Previous to the experiment, the animal was secreting free acid, and seven minutes before stunning, 2 mgm. of histamine were given subcutaneously. The animal was rendered unconscious by a blow delivered to the frontal region, exhibited decerebrate rigidity, etc., and lived for 40 minutes. A second dose of 2 mgm. of histamine was given 25 minutes after the animal became unconscious. No secretion whatever was produced in the 40-minute period following the blow. Other experiments are obviously unnecessary to convince anyone familiar with the physiology of gastric secretion that such a procedure causes an immediate cessation of gastric secretion.

Discussion. Cyanamin bichloride did not prove to be as satisfactory a reagent as we had anticipated. In the first place, it was eliminated in the gastric juice in too dilute a concentration for it to be definitely visible in the parietal cell. In the second place, it is not a delicate indicator of acidity, the blue color denoting acid reaction being obtained only at pH 3.0. Such being the case, the parietal cell could still be acid in reaction and not stain blue. Harvey and Bensley, and we, find the canalicular apparatus of the parietal cell to stain red using this indicator; however, it does not follow that the parietal cell is alkaline in reaction. On the other hand, it might be argued that since the pH of gastric juice is around 1.0,

if the parietal cell forms the free HCl, the parietal cell should stain blue to cyanamin which is blue at pH 3.0 But obviously one must keep in mind the fact that the parietal cells studied by us and others have been separated from the blood stream at least one and a half minutes, which quite probably is enough time for sufficient HCl to diffuse from the parietal cell to increase the pH to a value greater than 3.0. Also, it might be argued, although there is no good supporting evidence, that the HCl is secreted by the parietal cell at a pH greater than 3.0, the pH being decreased to 1.0 by absorption of water at the crypt of the gland. Harvey and Bensley believe that the secretion of the parietal cell is diluted at the level of the foveolae, but our observations, on using iron salts and neutral red, suggest concentration.

Our work using the iron salts as an indicator was disappointing. The toxicity of these salts makes observations unreliable unless the pouch is observed closely while the experiment is in progress, and then one does not feel certain but that the iron salts have killed a few susceptible cells, yielding the picture that one observes. We feel that the accumulation of sufficient iron in the parietal cells, following the introduction of Fitzgerald's solution, to give the Prussian blue reaction, is probably due to the depression of the activity of certain parietal cells; because with her solution and the dosage given, gastric secretion was practically always depressed. Harvey and Bensley certainly used too concentrated solutions of the iron salts. With Collip's solution, our failure to obtain the Prussian blue reaction in the parietal cells or gland lumina, was probably due to the rapid elimination of iron in minute amounts, insufficient to form the blue precipitate. But on successive injections sufficient iron salts accumulated in the mucus at the mouth of the crypt to yield the reaction. According to Stieglitz (1921), the duration of the circulation of free iron is quite brief, large quantities being taken out rapidly by the liver and kidneys. It was only by giving successive doses at intervals of from 15 to 30 minutes, that sufficient iron was eliminated by the stomach to be detected on the surface.

Neutral red proved to be an ideal indicator. Its small acid range, however, renders it impossible for us to state just how acid the cytoplasm of the parietal cell was under our experimental conditions. We can only state that it reacted acid to neutral red for a short time after excision from the pouch.

The results of two experiments; *a*, direct application to the mucosa, in which no staining of the gland resulted; and *b*, the immersion of the excised tissue, in which staining resulted, indicate that excision produces a profound change. When the active mucosa is intact, neutral red does not diffuse into the lumina of the tubules nor into the canaliculi; but when a portion of the mucosa is excised, the dye diffuses readily into the tubular

system. The cause of this is not evident; but it might probably be due to the loss of secretory pressure on excision. Another fact pointing in this same direction is that we observed a different staining reaction in the parietal cell when the dye was injected intravenously, and when the excised mucosa was immersed. In other words, the parietal cell stained in vivo gave a different picture from the parietal cell stained in vitro.

The three stages of reaction of the parietal cell observed by us may be explained as follows: 1, The initial or primary stage of acid reaction is due to free HCl formation in the cell.—The cell does not show a pH of 3.0 or less, as might be expected, because of rapid diffusion of acid from the cell. 2, The secondary stage of alkaline reaction is due to further and complete diffusion of acid from the cell and the cessation of acid formation due to excision (Hammett, 1915). 3, The tertiary stage of neutral and acid reaction is due to death of the cells.

The difference between our results and those of others who have investigated this problem is probably due to the fact that our tissue was obtained directly from the actively secreting mucosa, no unphysiological procedure preceding the excision of the tissue. In the work of others, unphysiological methods, which we know inhibit and abolish secretion, preceded the excision of tissue for study. In their experiments secretion was quite probably markedly inhibited or abolished even before they excised their tissue for study.

Our own methods are not ideal because we were forced to excise the tissue for study. But our method does shorten appreciably the length of time intervening between the moment of secretion and the moment of observation, making it possible to observe the reaction of the cell under more normal conditions than heretofore.

The regional activity of the gastric fundic mucosa as demonstrated by the regional elimination of both neutral red and iron salts, makes it apparent that uniformity in the staining reaction of different tubules should not be expected.

The findings on immersion of the tissue in neutral red might be explained on the basis of a pre-alkaline hypothesis for acid formation as follows: Assuming that at the moment of excision, the secretory pressure in the canalicular apparatus and gland lumina is immediately lost, it is possible that the hydrochloric acid formed in the crypts from the organic-HCl or alkaline precursor may diffuse inward along with neutral red and cause a transient acid reaction which is soon reversed by the alkaline secretion liberated from the parietal cells. If this were the case, one should expect the parietal cells near the foveolae to retain their crimson color longer than those cells near the tip of the fundus of the gland, which, however, is not the case. No uniform gradation in the persistence of the color in any particular group of parietal cells was observed.

It is also possible to interpret our findings in the following manner: In the cytoplasm of the parietal cell there is a weak acid reaction due to a partial dissociation of the H^+ and Cl^- from their precursors, and complete dissociation to the final extent only occurs in the canalicular apparatus or at the surface film separating the cytoplasm of the cell from the lumen of the canaliculus. In this case we have a mechanism very similar to that which takes place in the amoeba. Such an hypothesis finds support in our observations and does not require an additional explanation, as does the hypothesis of Harvey and Bensley, of why the HCl is dissociated only after it reaches the foveolae.

Until recently, biologists have generally held that any considerable acidity was extremely detrimental to the cell. The high concentration of acid found in the stomach they felt could not be produced within living cells. Several recent observations indicate that there may be a degree of acidity in uninjured protoplasm not heretofore suspected. Scarth (1924), in a study of the H-ion concentration of the vacuoles in a multinucleate amoeba, found the reaction within the vacuoles to be variable, some acid, others alkaline. Using neutral red as an indicator, he was able to observe the acidity in certain vacuoles increase until the neutral red assumed a deep purple. (The National Anilin and Chemical Company in their price list give the pH for the magenta color of neutral red as pH 0.0.) Scarth states that "the hydrogen ion concentration of a vacuole can be no criterion of that of the protoplasm that surrounds it—may indeed be such as applied externally would be lethal." While the acidity of the vacuoles in the amoeba must result from the activity of the surrounding protoplasm, nevertheless the protoplasm is sharply delimited as regards acidity. By comparison, the intracellular canalicular apparatus of the parietal cell may be regarded as an interconnecting series of vacuoles possessing a permanent outlet into the gland lumen. Also, Rous (1925), in his series of studies on the relative reaction of living mammalian tissues, has noted that the granules of certain macrophages are acid to bromthymol blue, a finding that indicates an acidity at least as great as pH 3.0. Further, it has been found that some living tissues are not injured when exposed directly to the acid contents of a living mammalian stomach. Dragstedt (1924) found that the decapsulated spleen and kidney transplanted into the stomach are not digested, provided care is taken to maintain their normal blood supply. The argument that the hydrochloric acid of the gastric juice is made by the parietal cell in some combined form which is alkaline in reaction, because free HCl would certainly destroy the cell, loses weight in view of these new facts.

Actual visual observation of the intact secreting parietal cell with suitable indicators of reaction, we believe constitutes the sole solution of this problem.

SUMMARY

The methods used by others for the study of the site of formation of the hydrochloric acid of the gastric secretion have been applied to Pawlow pouch dogs in which the effect of the agent on gastric secretion could be observed and a portion of the gastric mucosa could be removed and examined with a minimum loss of time. Iron salts should be used with caution for physiological purposes, because of their toxicity. The observations of Harvey and Bensley using cyanamin are confirmed, but we observe a primary stage following excision of the mucosa for examination, during which the cytoplasm of the parietal cell stains acid to neutral red. Two other succeeding stages are described confirmatory of those described by Harvey and Bensley.

We can conclude that under our experimental conditions the canaliculi and cytoplasm of the active parietal cell were acid to neutral red and alkaline to cyanamin; i.e., somewhere between pH 3.0 and pH 6.8.

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THE HEAT LIBERATED BY THE BEATING HEART

Fifth Communication

The Thermocardiogram of Limulus Polyphemus

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A consideration of the unique rôle that the heart of the king-crab plays in theoretical discussions of cardiac function has lead the author to determine the heat production of this organ in Limulus Polyphemus.[1]

1. After a few trials it was found that, if the usual care is exercised not to injure unduly the nerves and nerve ganglia, a preparation suitable for the determination of heat production may be made by isolating the middle five segments of the organ in a single piece. By bending the piece around upon itself the heart can be made to enclose an insert-thermopile, so that the ventral surface of the second and fourth segments of the piece comes in contact with the two sets of warm junctions on the two sides of the thermophile, while the two sets of cold junctions extend freely out in the space of the heart-chamber.

The problem of recording the tension exerted during the beats was met in several ways. First by tying silk ligatures to the stumps of the lateral arteries of the second and fourth segments, those of one side being attached to fixed points of the muscle holder, those of the opposite side attached first to the two corners of one side of a light and rigid isosceles triangle, the third angle of the triangle being in turn connected with the thread leading to the muscle-lever. A modification of this method was to insert through one artery stump a glass rod whose length equalled that of the segment and whose ends were hooked; the hook of each end was then made to protrude from the artery stumps and threads in turn connected the hooks with the muscle-lever. The circular fibers of the whole segment thus were kept at equal lengths and were enabled to exert their tensions upon the rods in an united action. Both these methods, however, were faulty in so far as the organ was forced to take on shapes and to suffer

[1] See Carlson (1905–1909), Meek (1909) and Nukado (1917) for original contributions to our knowledge of the finer structures and of the physiology of the Limulus heart; for a more recent and completer review of the literature, see R. Tigerstedt (1921).

pressures not normal to it. Indeed the heart did not seem to be able to maintain its beat when the second of these two methods was applied; it therefore had to be abandoned. It was obvious that a more nearly ideal method for recording the mechanical response of such an organ would be one that permitted it to be filled with fluid; by connecting the inner chamber with a manometer, one could then read directly the intracardiac pressure changes during the beat. By tying off the lateral arteries and the two ends of the heart-piece, by carefully disposing it upon supports, and finally by choosing a proper diastolic pressure a preparation was obtained that was quite free from leaks at the ostia and showed beautifully constant pulsations of the water in the manometer.[2]

The preparation was put in the usual dewar jar, it in turn was placed in an outer container kept (with ice) at temperatures from 1° to 3°C. The galvanometer used was a low resistance micro-volt sensitive d'Arsonval. The method of recording and analysing the temperature oscillations was the same as described by Snyder (1922) for recording and analysing the thermocardiogram of terrapin's ventricle. It may be recalled that it was shown in those experiments that the thermocardiograms only fell into marked phases, corresponding to what is known as initial and recovery phases of heat production, when the spontaneous contractions of the muscle (and thus the cardiac cycles) were sufficiently widely spaced to permit the period of the galvanometer to follow each phase separately. The best of such polyphasic thermocardiograms were gotten when the duration of the beat was about three minutes. In the limulus heart-preparations the interval between the spontaneous beats could not be increased (by the method used, that of lowering the temperature) beyond thirty seconds. The whole period of the galvanometer, however, was about twenty-four seconds. The thermocardiograms thus, as was found for terrapin hearts in such cases, could only represent the *total* temperature oscillation during the beat. This was taken as a measure of the *sum* of both *initial* and *recovery* heat-productions of a muscle-contraction.

In the experiments where tensions were recorded directly a calibrated sensitive isometric lever was employed which permitted an actual shortening of the circular bands of muscle fibers in the heart-wall, that was probably never more than 1 mm., and usually as little as 0.5 mm., an amount representing from 3 to 5 per cent of the muscle length or about 1.3×10^{-5} cal. of work and from $\frac{1}{3}\frac{1}{5}$ to $\frac{1}{6}\frac{1}{6}$ of the total energy of the muscle contraction.

2. In the results shown in tables 1 and 2 the values of tension and heat production given are the averages of a series of thermocardiograms, so

[2] The writer is indebted to one of his students, Mr. John Hall Kupp, for assistance in making and setting up the apparatus used in the latter two methods, also for assistance in carrying out some of the experiments.

long as the series included no individual data of marked variation. Variation did occur with the time, or age of the preparation, as is shown by the successive averages tabulated in the second and third experiments here given. The tension is recorded in the tables as T, which represents the maximum isometric tension exerted during the heart-beat per gram muscle. The oscillation of temperature for the beat is determined from the theoretical difference of temperature represented by the galvanometer deflection; this number times the specific heat of muscle (taken as 0.83) represents the calories of heat produced for each gram of muscle, and when multiplied by 10^5 is put down in the table under H_t. The ratio of the total

TABLE 1

Total heat production by the direct tension method

	HEART NUMBER AND THERMOCARDIOGRAM NUMBER	TEMPERATURE	T	H_t	H_t/T
		°C.			
I*	No. 1, 11 beats	5.2	7.96	48.1	6.04
	No. 2, 11 beats	5.2	8.49	71.6	8.46
	No. 3, 7 beats	5.2	8.18	64.3	7.86
	No. 4, 11 beats	5.2	8.87	53.1	5.99
Total averages			8.37	59.2	7.07
II†	No. 1. 2:15 p.m. 25 beats	3.0	26.7	25.0	9.40
	No. 2. 3:30 p.m. 11 beats	3.2	28.8	25.5	0.887
	No. 3. 4:00 p.m. 5 beats	3.4	26.3	23.9	0.910
	No. 4. 8:00 p.m. 8 beats	4.8	19.8	40.6	2.05
	No. 5. 9:10 p.m. 6 beats	5.2	19.2	32.2	1.68

* *Experiment of viii/10/25.* Limulus polyphemus, ♀. Direct tension method, ligatures to artery stumps. Weight of heart segments, 1.32 grams. Duration of beats, 28.2 to 29.6 seconds each. Temperature of heart chamber 5.2°C. Time of observations, from 2:40 p.m. to 5:20 p.m.

† *Experiment of viii/12/25.* Limulus polyphemus ♂. Direct tension or ligature method. Weight of segments, 0.79 gram. Duration of beats, 23 to 24 seconds each. Temperature given is that of heart chamber.

heat to the tension also appears in the tables. (For these calculations and the literature, see Snyder, 1922, also Snyder and Gemmill, 1925.) The segments of the heart that actually exerted the tension were weighed in each case; this weight as well as the temperatures of the heart-chamber will be found in the tables or the protocols.

The results especially of the second experiment show such great variation of the ratios H_t/T that one suspects that a serious error of technique is involved. As already pointed out, the method where the tension is transmitted through the segmental arterial stumps to the muscle lever is faulty in that all the circular bands of fibers can not register their united tensions directly upon the tension recorder. At the same time the inter-

mediate bands probably shorten more than those in the region of the artery stumps and thus do an unrecorded quantity of work. The third experiment (September 8), wherein the pressure manometer method was used, obviates all these technical errors. The results of this experiment, therefore, will be used in the following discussion.

3. As has already been discussed in previous papers of this series (1922, 1923) recovery heat as well as initial heat is probably complete with each heart-beat under favorable conditions. Now since it is known that

TABLE 2

Total heat production by the manometer pressure method

Experiment of ix/8/25. Limulus p., ♀. The direct pressure method is here used. The heart piece is filled with filtered sea-water and the intracardiac pressures are directly read off from the manometer. The temperature given is that of the heart chamber. The preparation was mounted in the chamber by 12:30 p.m.; the rate of beat continued too fast until 3:35 when for the rest of the experiment it was 12.5 to 16.5 seconds duration to the beat,—a rate still faster than desirable. The weight of the active segments was 1.30 gram. The values of T in the table and therefore also H_t/T are reckoned not per unit weight but for unit length of muscle, from the observed absolute systolic pressure.*

HOUR RECORD NUMBER		TEMPERATURE	T	H_t	H_t/T
		°C.			
3:35	I-b	2.8	1.34	12.6	9.4
3:48	I-c	2.7	1.23	12.6	10.2
4:15	I-d	2.6	1.18	15.1	12.8
4:45	I-e	2.6	1.03	13.8	13.4
4:55	I-h	2.8	1.03	11.3	11.0
5:40	II-i	3.0	3.46	73.5	21.2
5:47	II-j	3.0	3.37	67.0	19.8
7:00	III-k	3.4	2.26	21.4	9.46
7:30	III-l	3.6	2.88	13.8	4.79
7.45	III-m	3.8	2.74	25.2	9.21
8:20	III-n	4.0	2.52	13.8	5.48

* The heart of this animal is nearly cylindrical. The tensions therefore could be calculated from the observed pressures by the formula $p = \frac{T}{R}$ (see Walker, 1922) where p = pressure in grams per cm.^{2}, T = tension in grams, and R = radius.

the heats of these two stages tend to bear a constant ratio to one another especially in muscles such as frog skeletal, where conditions permit of greater accuracy of determination, it must follow that there is a tendency for the ratio of initial to total heat also to be constant.

In the fourth communication of this series of papers (1923) it was pointed out that the ratio of the initial to total heat production for terrapin ventricle is 1:4.33, a ratio based upon observed data that had been previously reported (1922).

It has been shown by Nukado (1917) that in spite of its gross morphological difference Limulus heart physiologically is much like the heart of vertebrates. Assuming this to be true one is justified, at least until the observed

TABLE 3

Comparing H_i/T and H_i/TW ratios for three different muscle organs

KIND OF MUSCLE	EXPERIMENT NUMBER	TEMPERATURE OF MUSCLE	WEIGHT OF MUSCLE (W)	TENSION* (T)	CALORIES INITIAL HEAT $p \times 10^5$ (H_i)	(H_i/T)	(H_i/TW)
		°C.	grams	grams			
Limulus heart; H_i is calculated from the observed total heat, on the ratio, 1:4.33. Experiment of ix/8/25 T = tension per centimeter length of muscle	I-b	2.8	1.30	1.34	2.81	2.17	
	I-c	2.7		1.23	2.81	2.36	
	I-d	2.6		1.18	3.49	2.85	
	I-e	2.6		1.03	3.18	3.10	
	I-h	2.8		1.03	2.61	2.54	
	II-i	3.0		3.46	16.9	4.90	
	II-j	3.0		3.37	15.4	4.58	
	III-k	3.4		2.26	4.88	2.18	
	III-l	3.6		2.88	3.19	1.10	
	III-m	3.8		2.74	5.81	2.12	
	III-n	4.0		2.52	3.19	1.26	
Average				2.09	5.84	2.79	2.15
Terrapin ventricles, 1921; d'Arsonval galvanometer; H_i, observed	1	10.6	1.45	19.8	14.9	0.75	0.52
	2	9.0	2.00	14.0	54.7	3.92	1.96
	3	8.0	2.70	9.5	36.1	3.84	1.42
	4	12.5	3.70	10.2	65.6	6.44	1.74
	5a	7.5	1.55	29.0	15.7	0.52	0.33
	5b	9.0	1.55	12.9	13.2	1.02	0.66
Average						2.74	1.10
Frogs' gastrocnemius muscles, 1925; thread galvanometer; H_i, observed	1	5.9	1.31	210.0	499	2.37	1.8
	2	4.7	1.83	75.6	278	3.67	2.0
	3	6.4	1.01	284.8	578	2.03	2.0
	4	3.3	1.78	34.9	149	4.27	2.4
	5		0.75	412.6	838	2.03	2.7
	6	23.0	1.15	608.6	1,608	2.64	2.3
Average						2.83	2.2

* Tension is stated in grams per gram muscle in every case except Limulus heart where it represents tension in grams per centimeter length of muscle fibers.

data are at hand, in calculating the initial heat (H_i) from the known total heat upon the basis of the ratio known for terrapin heart. Accordingly the values of H_i and H_i/T have been calculated for Limulus heart from the data of the experiment of September 8, and have been put in table 3

for comparison with similar ratios calculated from the *observed* data of other muscles.

In table 3 the ratio H_i/T states the number of calories of heat $\times 10^5$ produced per each gram tension per cm. length (Limulus) or gram weight (terrapin ventricle and frog gastrocnemius) of muscle. Comparing the values of this ratio for the various periods of the experiment on the Limulus heart one notes at once that the order of magnitude of the value as well as the range of deviation is much like those found for the two other muscles.

As explanation of the deviation in the H_i/T values one turns to possible uncontrolled as well as controlled variables in the conditions of the experiments. Of the latter it is obvious that temperature is not a contributing cause. Of the former one thinks of possible variations in the ease with which the deeper lying layers of tissue could be supplied with oyygen and relieved of CO_2. The conditions of these experiments, aimed as they are at the determination of small temperature changes, do not permit of maintaining a circulation of an adequate perfusing fluid through the tissues of the organ, hence the thicker the layers of cells to be supplied the greater the oxygen-want, etc. In other words, the surviving organs used in these experiments depend entirely upon the process of diffusion from the air in the muscle chamber for gaseous exchange to and from the innermost parts of their living substance. They therefore become asphyxiated directly proportional to their volumes. But neither the volume nor the thickness of the muscle masses was determined in these experiments. The weights of the active masses however were recorded, and since, density remaining constant, volume varies directly with weight,[3] we may substitute weight for volume and attempt a correction for the diffusion factor. This has been done and the values will be found in table 3 under the expression, H/TW, where W represents weight. The data for terrapin ventricle (1922) and also for gastrocnemius muscle (already published, 1925) have been treated thus also and appear in the lower half of the column.

Whether this improves the uniformity of the Limulus ratios cannot be stated from the one heart, but among the terrapin hearts a distinct betterment of the ratio is thus obtained, the disparity of the extremes passing from 1:12 to 1:6; likewise for the gastrocnemius pairs of muscles one notes a lessening of the disparity, the extremes passing from 1:2 to one of 1:1.5. It is further noted that the ratio of H_i/TW for the Limulus heart has very nearly the same value as the average of that ratio for the gastrocnemius muscle. The introduction of the weight (volume) factor into the ratio

[3] For example, the volume of a sphere of a substance whose density is constant may be said to vary directly as its weight. That is, since Vol. $= kr^3 = \frac{W}{D}$, $r^3 = \frac{W}{kD}$; assuming the density remains constant we may put $kD = K$ and $r^3 = W/K$, whence the relative value of W varies directly with the cube of the radius in case of spheres.

thus seems to justify the assumption that the deviations in the H_i/T value in all these experiments is in large part due to varying uncontrolled degrees of asphyxiation to which the muscles are unavoidably subjected.

In spite of the divergence of individual values it is remarkable that the average values of the ratio of H_i/TW for Limulus heart is so nearly of the same order of magnitude as it is for both terrapin ventricles and frogs' gastrocnemius muscles. This similarity is all the more striking when we put together the results of individual experiments where the values of T and H_i are themselves extremely divergent. Thus:

	W	T*	H_i	H_i/T	H_i/TW
Limulus heart (averages—ix/8).....	1.30	2.09	5.84	2.79	2.15
Terrapin heart, no. 2.....	2.00	14.0	54.7	3.92	1.96
Frog gastrocnemius muscle, no. 4.....	1.78	34.9	149.0	4.27	2.40
Frog gastrocnemius muscle no. 6.....	1.15	608.6	1608.0	2.64	2.30

* Tension is stated in grams per gram muscle in every case except Limulus heart where it represents tension in grams per centimeter length of muscle fibers.

Both tension and initial heat increase here more than 30,000 per cent yet their ratios vary less than 100 per cent.

4. It is concluded that, in spite of the sources of error met with in harnessing Limulus heart to thermophile and in maintaining a steady state for long enough periods, the observations on its heat production reported in the foregoing pages are an approximation to the true heat given off in the normal heart,—that each centimeter length of fibers in the circular layer for each gram tension exerted at each beat produces a total heat somewhere between 70 and 200 with an average of 115 microcalories, and that the corresponding initial or anaerobic moiety of this heat lies probably somewhere between 10 and 50 averaging about 26 microcalories.

Finally the source and manner of production of this initial heat in Limulus heart may not be so very different from that of terrapin ventricle (1922) which was found to vary from 5 to 64 giving an average of 27 microcalories, nor from that found for frogs gastrocnemius (1925) which varied from 20 to 43 giving an average of 28 microcalories.[4]

[4] In another place (1923) the author has called attention to the data for sartorius muscle. From the sources there given one can roughly calculate values expressing the heat production for this muscle in the same terms as are used above. The weights of the muscle pairs are given as varying from 0.13 to 0.24 gram, the mean of which is 0.185; the tension they exerted varied from 32 to 76 gram, giving a mean value of 54 grams. From these figures the value of T is 54/0.185, or 292 grams. The average initial heat is finally given as 0.0074 cal. per gram muscle, and the average total heat as 0.01776 cal. The total heat per gram tension (H_t/T) then may be put as 0.01776/292, or 61 microcalories, and the initial heat per gram tension (H_i/T) may be put as 0.0074/292, or 25 microcalories.

This similarity in the quantity of heat production among these very dissimilar organs is probably not due only to the fact that the fundamental contractile substance in all three organs is physiologically the same, but also to the fact that under the conditions of the experiments all three organs were responding to single, simple stimuli,—the two hearts to the normal intrinsic stimuli to beat, the gastrocnemius muscles to single induction shocks applied to the attached nerves and therefore also responding to the momentary nerve impulses falling simultaneously on all the fibers of the muscle.

On the basis of tetanic-like electrocardiograms that he obtained from the heart of L. polyphemus, P. Hoffman (1911) argued that the response of Limulus heart must be more like the response of skeletal muscle to voluntary innervation, and therefore that the nerve impulses supplied normally by the heart ganglion must also be of tetanic character. Observations upon another species of Limulus, and probably under more favorable conditions, led Nukado (1917) to deny the tetanic character of the normal Limulus electrocardiogram and to conclude that the intrinsic stimulus must be essentially of the same character as it is for vertebrate hearts.

The results of the present paper, showing as they do that the heat production in the beating Limulus heart is of the same order of magnitude as it is for beating terrapin ventricle and for frogs' gastrocnemius muscle when stimulated through its nerve by single shocks, would seem to favor the view that the normal stimulus to beat in Limulus heart whatever and wherever its origin may be is of the momentary, single volley type rather than the prolonged tetanic type of stimulation.

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Studies on the cardiac output of the dog. E. K. Marshall, Jr.

This investigation is a continuation of that reported a year ago (This Journal, lxxii, 192). A group of five dogs has been studied for periods up to 2 years. Under resting conditions, the minute volume of the heart in two male dogs has been constant within 10 per cent of the mean, in two female dogs the variations have been much greater. One animal followed throughout pregnancy has shown a greater variation than the males and a much higher output than any of the other dogs. It is suggested that the greater variability of female is related to the estrus cycle. No correlation between pulse changes and minute volume is apparent in four of the animals, in the pregnant dog there is an almost perfect correlation between pulse and minute volume. No general correlation between oxygen consumption or respiratory minute volume and the cardiac output has been seen. The mean minute volumes per square meter of body surface are 3.39, 2.64, 2.66, 2.76 and in the pregnant female 4.02 liters.

Muscular activity involved in shivering produced by chilling causes a marked increase in systolic output. Breathing air containing 7 to 8 per cent carbon dioxide causes a definite decrease in cardiac output per minute, with a marked increase in respiratory minute volume and an increase in hydrogen ion concentration of the blood. With 5 per cent carbon dioxide the decrease observed is less than the experimental error.

The proof of a humoral mechanism for pancreatic secretion. A. C. Ivy.

The entire tail of the pancreas has been transplanted subcutaneously into the mammary gland by the same method that Ivy and Farrell used to transplant a pouch of the stomach subcutaneously.

Such a pancreas transplant secretes a continuous secretion and is definitely excited to secrete after a meal, 6 cc. of juice having been collected in 1 hour. This fact definitely establishes a humoral mechanism for pancreatic secretion. Tenth normal HCl, water and secretin (intravenously) excites the pancreatic transplant. Three-tenths per cent $NaHCO_3$ does not stimulate as much as so much water, hence we believe $NaHCO_3$ inhibits slightly. These dogs have been kept on a diet of lean meat, olive oil and starch, each daily for two weeks and the secretions collected and studied for enzymes and solids. The secretion varied in quantity, but not beyond the normal variations in quality. From analyses of the secretion excited by different agents, it appears that the resulting secretion is always the same within normal variations. This observation argues strongly, but not conclusively, in favor of a hormone being the exciting agent concerned in the humoral mechanism.

An experimental study of reduced hepatic function. FRANK C. MANN and JESSE L. BOLLMAN.

We have previously described a method for producing a permanent decrease in the hepatic tissue of the dog. The success of the method depends on the discovery that the enormous regenerative power of the liver, whereby it is quickly restored to approximately normal size if damaged by poisons or if partially removed surgically, depends almost wholly on an intact portal circulation, that is, this regenerative power is almost lost in an animal after an Eck fistula has been established. It is thus possible to establish an Eck fistula and after a few weeks permanently remove various amounts of the hepatic tissue. We have made an intensive study of a large series of animals with permanently reduced hepatic tissue, in order to determine whether there were any specific changes characteristic of the condition, and whether the current tests of hepatic function as used clinically, would show evidence of reduced hepatic function. It was most difficult to show by any means that animals with markedly reduced hepatic tissue suffered a reduction of hepatic function as well. The test of function employed clinically did not differentiate a normal animal and one with less than 15 per cent of hepatic tissue. The positive findings of most interest were the production of a slight bilirubinemia occurring within a few hours after partial removal of the liver, which usually disappeared from three to five days later, and the appearance of the uric acid in the blood and its excretion in large amounts in the urine.

Electrical conductivity, electrical potential and hydrogen ion concentration measurements on the submaxillary gland of the dog recorded with continuous photographic methods. DETLEV W. BRONK and ROBERT GESELL.

In this investigation we have followed, by means of continuous photographic records, the electrical conductivity, C_H of the venous blood, electrical potential variations, and rate of secretion of the submaxillary gland of the dog during functional activity.

Two methods have been developed for obtaining continuous records of electrical conductivity. In one case a string galvanometer is used as a vibration galvanometer in an A. C. Wheatstone bridge. The second method comprises the usual A. C. bridge circuit with the primary of an audio frequency transformer in the bridge proper. The secondary of this transformer is the input of a three stage vacuum tube amplifier, the last tube of which is one arm of a D. C. Wheatstone bridge. The recording galvanometer—either string or D'Arsonval—is in this second bridge circuit, the deflections being proportional to the change in conductivity of the gland. The C_H of the blood was determined by the method of Gesell and Hertzman.[1]

The net electrical conductivity across the gland decreases whenever there is sufficient activity to produce secretion. Stimulation of the gland with Wharton's duct occluded or with an impaired blood supply causes a greater than normal decrease in conductivity. Secretory activity of the gland causes an increase in the C_H of the venous blood. Decreased blood supply during secretion or secretion against high pressure results in an increase in C_H which is larger than that resulting from secretion under normal conditions.

[1] Gesell, R. and A. B. Hertzman, Proc. Soc. Exper. Biol. and Med., 1925, xxii, 298.

Continuous records of arterial, venous and cerebrospinal fluid C_H in relation to pulmonary ventilation. ROBERT GESELL and ALRICK B. HERTZMAN. With the use of continuous methods previously described[1] changes in the hydrogen ion concentration of the arterial and venous blood and cerebrospinal fluid were studied under a variety of conditions. Data were obtained supporting the view that the hydrogen ion concentration of the arterial blood bears no causal relationship to pulmonary ventilation.[2]

Hemorrhage elicited decreased hydrogen ion concentration of the arterial blood and increased pulmonary ventilation. Re-injection was followed by increased hydrogen ion concentration and decreased pulmonary ventilation. Comparable changes in the hydrogen ion concentration of the blood were obtained with hemorrhage and injection during a constant artificial pulmonary ventilation, indicating the importance of volume-flow of blood.

Administration of air low in oxygen produced a sharp fall in the hydrogen ion concentration of both arterial and venous blood accompanied by increased pulmonary ventilation. Comparable changes in the hydrogen ion concentration were obtained when the gases were administered by constant pulmonary ventilation, illustrating the effects of reduction and oxidation of hemoglobin.

Mechanical asphyxia during constant pulmonary ventilation with room air led to a slowly developing acidity of the arterial blood and an early cessation of apnea. Similar mechanical asphyxia during similar administration of pure oxygen led to a rapid increase in the hydrogen ion concentration and a delayed cessation of apnea

Provided acidity is the major factor controlling respiration the lack of correspondence between blood C_H and pulmonary ventilation may find its explanation in the lack of correspondence between blood and tissue acidity. Since the cerebro-spinal fluid is separated from the blood by a membrane its reaction may give some indication of the changes in acidity occurring within the tissue cells. Records of the effect of injection of $NaHCO_3$ support the view that the tissues may turn acid on the injection of a base. Conversely, the administration of NH_4Cl may lead to increased acidity of the arterial and venous blood with decreased acidity of the cerebro-spinal fluid. Sodium cyanide in large injections may lead to increased alkalinity of the arterial blood and increased acidity of the cerebrospinal fluid.

The effect of a standard exercise upon the oxygen consumption of normal, overweight and chronically ill individuals. BYRON D. BOWEN and MABEL E. CARMER.

A comparative study of the oxygen consumption during rest and during an exercise was made in the following subjects—seven normal, twenty-one obese and overweight, twenty diabetic and fifteen with miscellaneous diseases. The exercise consisted in having the subject raise his legs rhythmically and alternately to the beat of a metronome so that his toes touched a string thirty inches above the plane on which he was lying.

The average percentage increases in metabolism according to surface area of the subjects who were thin or of normal weight, and obese or overweight, both diabetic and non-diabetic, were respectively plus 121.9 and

[1] Gesell, R. and A. B. Hertzman, Proc. Soc. Exper. Biol. and Med., 1925, xxii, 298.
[2] Gesell, R. This Journal, 1923, lxvi, 5, Physiol. Rev., 1925, v, 551.

plus 124.4, indicating that as a group obese people are no more economical of their energy than others. Different subjects show a wide but constant variation. With but few exceptions the exercise metabolism was high if the basal metabolism was increased and low if the basal metabolism was decreased. Duplicate tests were made on fourteen of the subjects, four of whom had an inconstancy in their basal metabolism with a relative inconstancy in their exercise metabolism. The basal-exercise metabolism ratio appeared to be an individual constant but varied widely in the different subjects.

The influence of testes on metabolism. E. P. BUGBEE and A. SIMOND.

Basal metabolism determinations were made twice a day on a male dog and on a female of nearly the same age and weight. After having established the normal basal metabolic rates, the male dog was castrated and the female kept as a normal control. The basal metabolism was determined at frequent intervals until 16 months after castration.

The castrated male showed a reduction in metabolism of 44 per cent, in terms of calories per hour per square meter, and the female at the same time showed a reduction of 35.4 per cent. The difference between the two dogs was not great enough to show that castration in itself had reduced the basal metabolic rate.

The opinion is expressed that, under the experimental conditions, other factors played a greater rôle than did castration in causing a reduction in the basal metabolic rate. Other factors suggested are the following: lack of psychic stimulation, lack of muscular exercise, adaptation of the nervous system and endocrine gland system to a life calling for less metabolism than in an active normal life.

Studies in experimental diabetes insipidus. HELEN BOURQUIN.

Diabetes insipidus has been produced in dogs by exposing the region of the hypophysis and directing an electric cautery into the mammillary bodies. That the effective lesion is injury to the mammillary bodies has been demonstrated by histological examination of the brain stem of nine dogs sacrificed while diabetic and of four others, which were not diabetic but were injured in the region of the base of hypophysis. The lesion common to all of the first group was injury of the mammillary bodies. In four that was the only lesion. The lesions in the latter group were in the tuber cinereum of two, lateral to the mammillary bodies of two.

Dogs can be rendered diabetic repeatedly in this way. If, however, the lesion is severe a diabetes already established is immediately stopped and cannot again be produced. Histological examination of the brain stem of eight animals subjected to this type of experiment showed complete destruction of the mammillary bodies. Diabetes insipidus is therefore an irritative and not a deficiency phenomenon.

It is entirely independent of the hypophysis. A group of four dogs was hypophysectomized and typical diabetes produced. In a second group of four dogs hypophysectomy was successfully performed without causing diabetes insipidus.

The cause of the polyuria is still undetermined. It is not a primary thirst phenomenon as some claim for typical diabetes is produced in dogs deprived of water after the operation. It is not due to an increase in blood chlorides, as others claim, for in 14 of 16 dogs, in which chlorides were

determined under standard conditions before and after diabetes insipidus was produced the diabetic blood contained the same amount as did the normal blood.

There is some evidence that the irritation causes something to be eliminated into the blood, which the kidneys can excrete, but cannot concentrate readily. Experiments are under way to test this hypothesis.

Note on a rapid and simple method of preparing a highly active pancreatic secretin solution. ARNO B. LUCKHARDT, O. W. BARLOW and MYRON WEAVER.

The first 100 to 150 cm. of dog's intestine are excised and the intraintestinal contents removed by passing a stream of tap water through the isolated loop. One hundred to 150 cc. of 0.2 per cent to 0.4 per cent HCl acid are now introduced into the loop and the ends closed with hemostats. The loop with acid contents is either incubated at body temperature or allowed to remain at room temperature for 10, 30 or 45 minutes. Ten minutes' extraction at room temperature usually suffices. At the end of that time the clear or straw colored content of the loop is filtered through cotton (or filter paper) to remove small flecks of desquamated mucosa. The filtrate is now ready for intravenous injection. On intravenous injection of 5 to 10 cc. (10 to 14 kgm. dog under barbital-sodium anesthesia) a copious secretion of pancreatic juice ensues comparable often in quantity to that prepared by the more prolonged method of Bayliss and Starling. The earlier such extractions are carried out following excision of the loop of gut from the animal, the less vasodilatation is seen following intravenous injection. We have prepared some extracts with pronounced secretagogue action which were virtually devoid of vasodilatins. Both vasodilatin and secretin dialyze through celloidin sacs. When kept in brown bottles under toluol in the ice box, the preparations made by the method just described seem to lose their secretagogue action more rapidly than those preparations made from the mucous membrane by the usual process of maceration, grinding with sand and boiling with 0.4 per cent HCl. The vasodilatin action persists, however, indicating anew that the secretagogue action of pancreatic secretin solutions is not due solely to the vasodilatins almost invariably present in them.

Studies on the pathological physiology of experimental gangrenous pancreatitis. LUCIA E. TOWER.

Acute hemorrhagic pancreatitis was produced in dogs by complete ligation of the blood supply to different amounts of the pancreas. In some additional damage was done by crushing the gland or injecting bile through the pancreatic duct. Attempts were made also to reproduce the characteristic toxemia of acute pancreatitis by placing excised pancreatic tissue in the abdomen, wrapped in the omentum. Careful bacteriological studies were made of the fresh gland and of the tissues and peritoneal exudate at autopsy. About fifty animals were used for these experiments.

All of the different pathological conditions characteristic of human cases of acute pancreatitis were produced by these methods. There was a wide variation in the symptomatology and pathology resulting in different animals from the same identical operative procedure.

Four of eleven animals (36 per cent) with one-third of the gland destroyed (i.e., all blood vessels tied off and the portion thus isolated left in situ) died in 24 to 72 hours of typical hemorrhagic pancreatitis.

Fourteen of twenty-five animals (56 per cent) with two-thirds of the pancreas destroyed (head and tail) died in 18 to 120 hours.

All animals (6) with more than two-thirds of the gland destroyed died in 48 hours or less. Unmistakable signs of a severe toxemia were present in all animals of these three groups except in 20 per cent of those with but one-third of the gland destroyed.

Of ten animals, six with one-third, three with two-thirds and one with the entire gland placed in the abdomen wrapped in the omentum, none died. In none were there evidences of a severe toxemia; in those with two-thirds or more allowed to autolyse in the omentum there were evidences of a moderate degree of indisposition. This indicates that while there is undoubtedly some toxemia from the absorption of products of autolysis of the pancreas, the fatal toxemia of acute pancreatitis must have some other basis for its explanation. This and other evidences gleaned from clinical and pathological studies lead to the suggestion that acute necrosis of the pancreas kills by virtue of the local action of the products of autolysis on the gastro-intestinal tract, presumably on the musculature of the gut and possibly also that of the splanchnic blood vessels. The toxemia produced would then be of the nature of that associated with a paralytic ileus. The remarkable similarity between the symptomatology of acute pancreatitis and acute intestinal obstruction, the fact that complications which would indicate a disturbance in gastro-intestinal motility are not infrequent in man and experimental animals, and the not infrequent occurrence of obstipation as a symptom in acute pancreatitis would all tend to support this view. In my own series of experiments three animals have died of intussusception in the jejunum, one of acute dilatation of the stomach, and dilatation of parts or all of the upper gastro-intestinal tract has practically been the rule at autopsies.

Attempts were made to produce a sterile pancreatitis. All of these failed (21 sterile autopsies). Organisms were always found in one or more of the cultures taken from the peritoneal exudate, the gangrenous gland, or the tissues.

Bacteriological analyses of sixteen samples of fresh pancreas tissue removed sterilely at operation showed the presence of bacteria in all but one. It would appear from this that bacteria are normally present in the gland, presumably in the ducts.

In general those organisms found in the fresh gland and in the tissues and exudate at autopsy were of a nonpathogenic variety and were those organisms common to the upper gastro-intestinal tract.

Although it was not possible, as was originally intended, to produce the typical toxemia of acute pancreatitis with the action of bacteria within the pancreas and other tissues eliminated as a factor, it was felt that their presence (except in the pancreas) was merely incidental to the extreme disturbance of vitality of the tissues of the abdomen which gave them an opportunity to gain a temporary foothold. The fact that organisms may be demonstrated in the peritoneal exudate of an animal well on the road to recovery, when there is no evidence whatever of peritonitis, would support this view. Whether or not the fatal toxemia is augmented by the presence of these organisms from absorption of products of their growth in the tissues it would be almost impossible to determine at present.

If the fatal factor in acute pancreatitis is of the nature of a paralytic ileus, this would open up the possibility of more effective treatment by

measures aimed at this condition. Experiments along these lines are at present in progress and will be reported later, as will also a group of experiments being conducted on the blood chemistry changes in acute pancreatitis.

Studies on the influence of physical work on the basal metabolism. ARTHUR H. STEINHAUS.

Dogs were trained for basal metabolism tests. The diet was adjusted to maintain body weight and then continued *without change* throughout the experiment. The basal metabolism was determined daily; 1st, during a period of caged life with practically no physical activity; 2nd, during a period of strenuous running on a motor-driven tread, and 3rd, during a post-exercise period of inactivity.

To date it is possible to report on the results from two dogs.

Dog 1 was caged from November 22, 1924 to March 28, 1925, and then exercised until April 24. The running was increased from two miles at the start up to twelve miles per day as rapidly as his training permitted. All of the work was done on a 22 per cent grade at a rate of a mile in 18 minutes. Observations through May 23 fall into the post-exercise period.

Dog 4 was caged March 24, 1925. Exercise was begun July 2 but because of very warm weather the exercise did not regularly exceed three miles per day until early September. Since then to date (December 26) with the exception of a thirteen-day no exercise period, he has run around 8 to 10 miles each day on a 22 per cent grade at the rate of a mile in fourteen minutes.

Summary of results

	AVERAGE WEIGHT	AVERAGE CALORIES PER HOUR	CALORIES PER KILO PER HOUR	PER CENT DIFFERENCE
	kilos			
Dog 1:				
Pre-exercise:				
15 tests between March 5 and 28	8.79	16.37	1.86	
Exercise:				
25 tests between March 29 and April 25	8.25	16.60	2.01	+8
Post-exercise:				
22 tests between April 26 and May 22	8.71	16.43	1.89	+1
Dog 4:				
Pre-exercise:				
15 tests between June 1 and July 2	17.09	25.18	1.47	
Exercise:				
23 tests between October 3 and November 4	17.51	24.30	1.38	−6.1
Post-exercise:				
11 tests between November 5 and 17	16.88	22.25	1.31	−12.2
After resumption of exercise:				
10 tests between December 17 and 26	14.81	19.61	1.32	−10.2

The data collected to date indicate that the basal metabolism is not increased by a period of intensive physical activity.

If one assumes that the weight lost in a period of training represents the inactive fat deposits, the increase in calories per kilo per hour in dog 1 becomes insignificant and the data on dog 4 may be interpreted to show a

lower metabolic rate in the muscles in the trained state. This may be due to better relaxation.

Of further interest is the fact that the metabolism of dog 4 decreased consistently throughout the experiment with seemingly no regard to the exercise factor.

It may be significant that the animals were confined indoors and caged throughout the experiment.

There is some indication in our work that a cool night in the kennels may cause a heightened basal rate which hangs over for several days. This is being investigated.

Changes induced by blood constituents (serum and cells) and by intima of blood vessels on the blood pressure response of pituitary extract. RICHARD WHITEHEAD.

It has been claimed that the dilator response resulting from repeated injection of pituitary extract is due to a reaction of the blood with the latter, resulting in the production of a dilator substance.

The present work was done in the attempt to determine the effect which individual blood constituents might have and in this way determine what part of the blood was responsible for the results reported.

Pituitary extract was mixed with serum, cells, and in another series of experiments with whole blood. The mixtures were kept at body temperature for varying periods. The drug was perfused through the veins and arteries in another series of experiments, before injection was made into the vein.

In none of these experiments was a fall of blood pressure obtained when the mixtures were subsequently injected intravenously into dogs, anesthetized with ether. The response after injecting serum or cells mixed with pituitary extract was not so great, however, as when untreated pituitary extract was used.

The results obtained tend to show that none of the separate blood constituents cause the conversion of pituitary extract into a dilator substance. The perfusion of the extract through the vessels before injection caused a "sensitization" of the former, as evidenced by the higher rise of blood pressure obtained after intravenous injection of this perfused preparation. The latter results are to be studied further.

A simultaneous study of the chlorides and non-protein nitrogen constituents of the sweat, urine and blood as affected by heat and work. G. A. TALBERT and E. E. HARRIS, J. R. FINKLE, S. H. SILVERS (by invitation).

While we are unable in all instances to show a consistent conformity to a rule, we believe that we are justified in the following conclusions.

In regard to the chlorides, our analysis reveals 3.2 to 6.5 mgm. of chlorides per cubic centimeter. The lowest concentration seems to be from heat sweat produced before the labor of the day is begun. The work sweat produced from the early morning labor will run uniformly higher, while that produced by heat at the end of a hard day's work will register higher still. On Sundays when the subject remained quiet for a few hours before sweating the samples yielded a high concentration; yet, the urine immediately following the sweating ran low in chlorides.

The total nitrogen showed a much more marked fluctuation. It varied from 0.338 to 1.625 mgm. per cubic centimeter. It showed, how-

ever, variations similar to those of the chlorides in comparing the morning and late afternoon samples. It differed, however, in that the work sweat was of a markedly lower concentration. On Sunday the total nitrogen ran high along with the chlorides.

Our experiments showed, as a very general rule, that the chlorides and the total nitrogen in the sweat vary directly, and not inversely as reported by Kittsteiner.

There is a quite marked tendency for the chlorides of the sweat and urine to vary directly on the days of hard labor. On the contrary, on Sundays, they run inversely as shown by the high concentration in the sweat and the low concentration in the urine.

On the contrary, a comparison of the total nitrogen in the sweat and in the urine shows the tendency of this constituent to vary inversely on working days and directly on Sundays.

The urea fluctuated in the sweat from 0.05 to 1.12 mgm. grams per cubic centimeter, and the ammonia from 0.04 to 0.211 mgm. per cubic centimeter. Our figures indicate on the whole a higher concentration of urea in the sweat when the subject is resting, while an increase of the ammonia is more manifest as a result of fatigue.

We discovered that heat sweat causes a rise in the specific gravity of the blood, and also a slight increase in its chlorides and in its total non-protein nitrogen.

The rôle of calcium in the coagulation of blood. MILO M. LOUCKS, introduced by F. H. SCOTT.

These studies on the relation of calcium to coagulation of blood were made to determine, first, whether or not calcium was essential to the first stage of coagulation; that is, the formation of so-called thrombin only, or for the entire clotting process; second, if a Ca free thrombin would produce coagulation, and third, to repeat the work of Mills and Guest[1] to determine whether or not calcium entered directly into the formation of fibrin and constituted a part of it.

Thrombins were prepared in three ways according to Howell, Rettger and Morawitz. These were of normal activity when prepared from normally formed fibrin or blood serum, but if these thrombin preparations were deprived of calcium by oxalate and excess oxalate removed by dialysis, they lost their activity. Their activity returned on the addition of calcium. The calcium could not be removed by dialysis. Moreover, an active thrombin could not be prepared from Ca free serum or calcium free fibrin. Calcium free tissue fibrinogen would not react with Ca free blood fibrinogen to produce fibrin.

The work of Mills and Guest was repeated, adding varying amounts of tissue extract to a given quantity of citrate plasma and $CaCl_2$. A constant relationship between Ca in the fibrin and total fibrin weight was not found. The calcium content of fibrin formed in this way was extremely variable and subsequent experiments showed that the Ca could be removed from the fibrin by prolonged washing with 0.9 per cent NaCl followed by distilled H_2O. This I think shows the Ca is not an essential constituent of the fibrin. The calcium determinations were made after ashing in an open flame, dissolving the ash in hydrochloric acid, bringing the solution

[1] Mills and Guest. This Journal, 1921, lxxvii, 395.

to a pH of 3.5 to 7.5 by means of acetate and precipitating with oxalate and titrating the calcium oxalate formed against potassium permanganate.

The metabolic rate of thyroparathyroidectomized dogs. FRED R. ISAACS (by invitation).

The purposes of this study of the metabolic rate of thyroparathyroidectomized dogs were: 1, to determine the general change in the rate of oxygen consumption following thyroparathyroidectomy; 2, to determine whether or not there is any change in the rate of oxygen consumption of such animals during the pre-tetany stage, 3, to determine whether or not the increase in the rate of oxygen consumption brought about by feeding desiccated thyroid to thyroparathyroidectomized dogs is accompanied by tetany; and 4, to determine whether or not the injection of guanidin carbonate into thyroparathyroidectomized dogs produces an increase in their rate of oxygen consumption.

The dogs were selected, trained to lie quiet during the test periods so as to produce basal conditions, and were given an adequate diet of bread, milk and liver. The tests were run on a Benedict-Sanborn metabolimeter. They were run at least sixteen hours after feeding and the dogs were allowed to lie quietly upon the table thirty minutes before the test readings were taken. The records of the oxygen consumption of each animal preceding thyroparathyroidectomy constitute a control for that animal. Complete thyroparathyroidectomy was performed upon each of six animals under morphine-ether anesthesia. The parathyroid tetany was controlled by the oral administration or intravenous injection of calcium lactate, only one dog dying of tetany before any post-operative records could be taken.

The rate of oxygen consumption was determined before, during and following the attacks of tetany. In all cases no appreciable change in metabolic rate was found in the periods when the animals were free from tetany. In the intermediate pre-tetany stage an increase in oxygen consumption was noted which increased during the progress of tetany, reached its maximum during the height of tetany and declined to normal shortly after the symptoms of tetany disappeared. This increase seemed to be a result rather than a cause of tetany. The oxygen consumption of all dogs reached a level below that of the pre-operative period after the attacks had become less frequent or had disappeared. This decrease can probably be attributed to the loss of thyroid tissue. Only one dog was fed desiccated thyroid and although the rate of oxygen consumption increased materially no tetany occurred. One of three dogs receiving guanidin showed an increase in metabolism but no symptoms of tetany. One showed a marked increase in the rate of oxygen consumption following the appearance of guanidin convulsions which were fatal. The other of the three dogs receiving guanidin showed no tetany or increase in oxygen consumption.

These results indicate that parathyroid tetany is not caused by changes resulting from the altered basal metabolic rate. The increased oxygen consumption during tetany was undoubtedly caused by the increased muscular activity. The general decrease after the tetany attacks had practically disappeared must have been due to the hypothyroidism. The results with guanidin are not inconsistent with the theory that guanidin is a factor in the production of parathyroid tetany.

The respiratory quotient of resting muscles. H. E. HIMWICH and W. B. CASTLE (introduced by C. K. DRINKER).

Previous observations of respiratory quotients have been made either on excised muscle or on the living animal where the muscle was not isolated. Our studies were on isolated muscle, still part of the living organism. Dogs were decerebrated and all the vessels of one lower limb except those of the gastrocnemius and flexor digitorum sublimis were tied off. Samples of arterial and venous blood were analyzed for O_2 and CO_2 by the methods of Van Slyke and Neill. The O_2 and CO_2 of expired air were determined with the Haldane-Henderson apparatus in order to calculate the respiratory quotient of the animal at the time the blood samples were being drawn. The average of 19 determinations of the respiratory quotient obtained from blood was 0.71 ± 0.10 and from air 0.80 ± 0.04. In spite of improvements in methods of preparing animals, collecting and analyzing blood and calculating the respiratory quotient in individual cases, the variations obtained from simultaneous samples of blood and air were large, the mean difference being 0.17. If it is permissible to take averages the following conclusions may be drawn:

1. The respiratory quotient of resting muscle *in situ*, under the conditions studied, is less than unity and is probably close to that of the body as a whole.

2. Resting muscle does not oxidize carbohydrate exclusively but also fat or protein or both.

The utilization of protein, carbohydrate and fat in hypoglycemia in diabetics requiring insulin; the glucose content of the blood plasma and corpuscles, and the effect of insulin and glucose on the acid-base balance of the blood. R. B. GIBSON, LILLIAN GREER and ADELAIDE BARER.

In the hypoglycemic state there is an increased utilization of fat and lessened oxidation of carbohydrate in 4 experiments and little or no change in 1 experiment as compared with higher or normal levels under very similar conditions. In 3 experiments hypoglycemic levels were not obtained. It would seem that, at high blood sugar levels, the metabolism is of the diabetic type; figures within the normal range are associated with an increased carbohydrate utilization, and at hypoglycemic levels the fat metabolism is augmented. The total energy metabolism is somewhat increased in hypoglycemia. Blood sugar levels are regarded as incidental to and not the cause of the metabolic changes. No anhydremia of the blood at lower sugar levels is indicated from gross hematokrit values either in the above cases or in other patients receiving insulin and glucose. While differences in plasma and corpuscle sugar as reported by Foshay obtain, such differences are not so great, and the hypoglycemic state is not associated with a disappearance of glucose from the red cells.

Figures are given for cases receiving pre-operative preparation with insulin and glucose in which there occurs a pronounced shifting of the acid-base balance further to the alkaline side. The effect is maintained even after long continued anesthesia and severe operative procedure. It may be explained by the recognized binding of phosphoric acid when insulin and glucose are available. Thus another factor which influences and helps to regulate the acid-base balance is presented.

On the occurrence and control of an anemia that follows total gastrectomy in dogs. J. I. FARRELL (by invitation).

The occurrence of anemia has been observed in three totally gastrectomized dogs, one dying at eight months, a second at seven months and a third now alive for over a year. The anemia was not discovered until two weeks prior to the death of the first dog. The third animal has now been studied closely for one year and the effect of various therapeutic procedures has been observed.

The sequence of events that occur in these dogs is as follows: From four to six months after gastrectomy the animal begins to lose weight and develop an anemia. This continues until between one-fourth and one-sixth of the original body weight is lost and the hemoglobin decreases to a value between 40 and 50. Anorexia develops shortly after the appearance of the anemia and gradually increases until the animal refuses food the two weeks prior to death.

The routine diet of these animals is meat (1.5 pounds, ground), bread (100 grams) and whole milk (1 to 3 pints, ad libitum).

Treatment was instituted in the second animal, but his condition had advanced beyond therapeutic control. Treatment was instituted in the third dog when the body weight was reduced from 19 K. to 17 K. and the Hb from 72 to 52. In this animal we have found that cod liver oil by mouth (8 cc. daily) and iron subcutaneously (ferric citrate, 0.1 gm., 3 times a week) will induce recovery. On cod liver oil alone body weight was maintained, but anemia developed. On iron alone no improvement whatever resulted. The feeding of liver or of milk acidified to the point of clotting was of no benefit.

The typical blood picture when the animal is anemic is as follows: r. b. c., 5 million (normal, 7 million); w. b. c., 15,000 (normal, or when not anemic, 22,000); Hb., 48 (normal, or when not anemic, 80); definite anisocytosis; polymorphs, 63 per cent; large lymphocytes, 10 per cent; small lymphocytes, 8 per cent; eosinophilic polymorphs, 11 per cent; monocytes, 3 per cent; meta-myelocytes, 5 per cent. We have never observed poikilocytosis or normoblasts. We have seen a few myelocytes. This picture denotes mild bone marrow irritation. It is of interest to note that as the dog becomes anemic the w. b. c. decrease. The fall in Hb precedes the decrease in the number of reds, the color index always being less than 1.0.

Estimation of blood corpuscle volume by the eriometer. W. F. EMMONS.

The eriometer is an instrument for measurement of the diameter of small objects by means of diffraction spectra, or coronae. It readily gives the average diameter of corpuscles in a dried smear of blood. Volume measurement of the corpuscles determined with the hematocrit or refractometer have been compared with the eriometer readings of diameter, and a constant relation has been found to exist between them. Consequently the volume can be read off dried smears directly by means of the eriometer.

The action of insulin on the domestic fowl. G. J. CASSIDY, S. DWORKIN and W. H. FINNEY.

The effects of insulin administration in fowls differ from those observed in mammals: convulsions do not occur, and the fall of blood sugar is accompanied by lowering of body temperature. A state simulating hibernation cannot be induced in fowls.

The rôle of platelets in mammalian blood coagulation. HUGH E. BURKE.

It has been found possible by the intravenous injection of Wooldridge's tissue-extract or "thrombokinase" to obtain blood which is platelet-free. This blood does not spontaneously clot on glass. It clots on the addition of thrombokinase.

By the use of cooled mammalian plasma it can be shown under the microscope that platelets, owing to adhesion to glass, suffer an abrupt disintegration and yield something which brings about deposition of strands of fibrin.

The effects of pH, light and other factors on the penetration of 2,-6,-dibromo phenol indophenol and other dyes into a living cell. MATILDA MOLDENHAUER BROOKS.

Valonia, a marine, one-celled alga, was placed in solutions of 2,-6,-dibromo phenol indophenol and the amount of dye found in the sap was measured colorimetrically when the external pH was varied; when the internal pH was varied; and when screens admitting light of different wave lengths were used. When the proportion of undissociated dye in the external solution was changed by changing the pH, the amount of dye in the sap of normal cells at equilibrium was proportionate to the amount of undissociated dye in the external solution. At a constant external pH, the amount was greater when the cell-sap was more acid, and less when the cell-sap was more alkaline. The dye was always found completely reduced in the sap. Penetration was greatest in diffuse light and least in darkness; when the plants were exposed to light of different wave lengths from ultra-violet to red, penetration decreased as the wave lengths increased.

Methylene blue penetrated into the sap in an oxidized form and the amount in the sap did not vary with external and internal changes in pH.

Other dyes gave less definite results.

The reduction potential of the sap was found to be between pH 16 and 18 subject to certain assumptions. That of the protoplasm was less definitely defined.

Studies in albuminuria. HILDING BERGLUND and WALTER SCRIVER.

Factors influencing the albumin output through diseased kidneys have been studied in cases of acute and chronic nephrosis as well as in animal experiments. Both serum-albumin and serum-globulin usually escape into the urine. Albuminuria without globulinuria may occur but is evidently rare. A high albumin to globulin ratio in the urine signifies a lesser degree of abnormal permeability than a low ratio. The urinary albumin to globulin ratio is always higher than 1. The urinary ratio is independent of the corresponding ratio of the plasma.

On a high protein diet the total protein output through the kidneys may become increased as much as three times above the output during a previous or following period of low protein diet. Increased elimination of urea after urea feeding has no similar effect on the amount of urinary protein.

This phenomenon does not indicate increased kidney damage. In fact, it indicates a greater amount of protein circulating through the kidneys. This statement is based upon observations on the concentration of the plasma proteins. The protein synthesis after high protein feeding is a rapid process. In three days the plasma proteins may increase 30 per cent.

The rôle of the suprarenal gland in the natural resistance of the rat to diphtheria toxin. DAVID L. BELDING and LELAND C. WYMAN.

An attempt was made to determine the influence of suprarenalectomy upon the mechanism of resistance of the albino rat to diphtheria toxin. It is known that double suprarenalectomy increases the sensitiveness of rats to a variety of poisonous drugs and toxins, and that rats have a high natural resistance to diphtheria toxin. Both suprarenal glands were removed in one operation by the posterior route and the control rats received the same operative procedure except the removal of the glands. The highest post-operative mortality in the suprarenalectomized animals occurred with fluctuations in room temperature, and rectal measurements indicated a lessened ability of the suprarenalectomized rats as compared with the controls to maintain their body temperature in a cold atmosphere. An approximation of the lethal dose of diphtheria toxin administered intraperitoneally for suprarenalectomized and control rats two to three weeks after operation showed that the controls were about two and one-half times as resistant to the toxin as the suprarenalectomized rats. The amount of diphtheria toxin in the blood four hours after an intraperitoneal injection of one M.L.D. per gram of body weight was measured by the Roemer intradermal method in guinea pigs. The suprarenalectomized rats had approximately 2.75 times as much free toxin in their blood as the control rats. These facts indicate that suprarenal deficiency renders less effective some factor which is responsible for the natural resistance of the normal rat to diphtheria toxin.

The nature and time relations of the compression sounds of Korotkov in man. HORACE MARSHALL KORNS.

1. In the normal man, and in patients with aortic regurgitation, the Korotkov compression sounds are composed of a series of regular vibrations of generically uniform character. The number of vibrations per sound varies from three to six. The vibration periods range from 0.0039 to 0.025 second (e.g., between 256 and 40 dv per second), and the duration varies from 0.041 second in a sound of four double vibrations to 0.1275 second in a sound of six double vibrations. The duration of a sound depends not only upon the number of component vibrations, but also upon the duration of component vibration periods. The period of the first vibration is always shorter than that of succeeding vibrations. On the basis of certain characteristic differences in period and amplitude the vibrations are divided into three groups which are designated as *introductory*, *main*, and *final*. The first *main* vibration is usually the one of greatest amplitude, but in some sounds the second is higher than the first, and in others the main vibrations are all of nearly equal amplitude. The loudest sounds invariably comprise 6 double vibrations of relatively long period, in consequence of which their pitch is comparatively low, and their duration maximal. However, sounds of 5 double vibrations are frequently more intense than sounds which comprise 6 double vibrations, and furthermore, low pitch is not a pre-requisite of intensity, for a relatively short average period often exists in conjunction with more than average intensity. During progressive decompression the pitch of the sounds shows a general tendency to fall slightly as their intensity increases. The employment of an unusually narrow cuff interferes seriously with the normal character of the sounds, but promotes the formation of murmurs. Frank murmurs

of appreciable magnitude and duration rarely occur when a cuff of the proper width is employed. Reduplication of the arterial sounds is an interesting phenomenon occasionally encountered.

2. The changes occurring in the form of the compression pulse during decompression are analyzed, and it is demonstrated that penetration of the compressed artery at successively lower arterial pressure levels determines progressively earlier incidence of the Korotkov sounds with respect to the initial rise of the compression pulse.

3. Contributory evidence is brought forward in support of the reasonable assumption that the mechanism which operates to produce the Korotkov sounds in man is identical with the water hammer action which Erlanger has found to be the cause of arterial sounds in animals. Furthermore, it is demonstrated that initiation of the hydraulic impact of water hammer is characterized by sound vibrations of relatively short period.

4. The similarity of the vibrations at all levels of decompressing pressure indicates that the Korotkov sounds originate in an impact at one fixed spot. There is no evidence that eddies or whorls contribute to sound production.

5. Division of the Korotkov sounds into so-called "phases" seems unwarranted. It is suggested that in this respect our clinical conception of the sounds is in need of revision.

Changes in rate of the denervated heart during bulbar anemia, after excision of the adrenals. HELEN C. COOMBS.

The changes in heart rate during bulbar anemia in cats before and after excision of the adrenals have been given in previous papers. In the present series of experiments these observations were repeated on the denervated heart. The results are given in the following table:

	HEART RATE BEFORE OCCLUSION	20 SECONDS AFTER OCCLUSION	60 TO 120 SECONDS AFTER OCCLUSION	20 SECONDS AFTER RELEASE
Control heart rate	204	104	228	228
Second occlusion after removal of stellates	228	164	240	200
Third occlusion after section of both vagi	204	232	288	240
Fourth occlusion following excision of both adrenals	168	176	180	172
Fifth occlusion, ten minutes later	154	166	174	158

Following the fifth occlusion, the heart rate remained at 158 for about half an hour, during which time artificial respiration was maintained. There was no return of medullary activity. At the end of this time, artificial respiration was discontinued. Care was taken in these experiments, to use only cats which were known to have been kept without food for at least twenty-four hours.

Since the heart, under these conditions, has been denervated, one would be inclined to attribute such relatively slight changes in heart rate as occur after removal of the adrenals to a redistribution of the blood. If this is the case, any redistribution of the blood other than occlusion of the head arteries, should produce a similar effect. In a number of experiments, therefore, the abdominal aorta was occluded at various times during the

experiment, to study the effect of this redistribution of blood. Normally, compression of the abdominal aorta is attended by compensatory slowing of the heart rate. In the fasting animal, with adrenals excised and heart denervated, there was usually a slight acceleration of heart rate on compression of the abdominal aorta; in fact, the change was quite comparable to the alteration in rate brought about by occlusion of the head arteries at such a time, as the following table shows:

	HEART RATE BEFORE OCCLUSION	20 SECONDS AFTER OCCLUSION	60 TO 120 SECONDS AFTER OCCLUSION	20 SECONDS AFTER RELEASE
Third occlusion after denervation of heart	190	224	260	236
Fourth occlusion following excision of both adrenals	152	158	162	154
Rate after 4th occlusion	154			
Rate on compression of abdominal aorta	164			
Rate on release of abdominal aorta	156			

No observations have as yet been made upon animals in which the adrenals were previously removed and an interval of recovery elapsed before heart rate was studied under these conditions.

The relation of insulin dosage to the blood sugar drop, together with some related phenomena. ERNEST L. SCOTT.

A series of fifty observations under each of the following conditions was made. Rabbits were used throughout.

a. Control series. No insulin at all was given but the animals were injected with the standard amount of isotonic sodium chloride solution immediately after the initial sample of blood was drawn.

b. One-sixteenth of a unit of Lilly's U20 Iletin was given per kilo of body weight. This was given as were all of the following in the standard amount of isotonic salt solution, 1 ml. per kilo.

c. One-eighth of a unit per kilo.

d. One-fourth of a unit per kilo.

e. One-half of a unit per kilo.

f. Three-fourths of a unit per kilo.

g. One unit per kilo.

In each experiment a sample of blood was obtained just before the subcutaneous injection and then $\frac{3}{4}$ of an hour, $1\frac{1}{2}$, $2\frac{1}{2}$ and $3\frac{1}{2}$ hours after the injection.

The blood sugar drop following the dosages studied was found to follow roughly the curve described by the formula $(y - 2)(x + 0.3) = -1.5$. y = blood sugar dosage; x = insulin dosage. No special significance is at present seen in the particular form of this equation and it is presented only as a convenient manner of describing the curve and to illustrate the possibility that a mathematical analysis may ultimately be applied. The equation does not hold for dosages lighter than those reported.

The maximum drop occurs at about $\frac{3}{4}$ of an hour after injection of the minimum dose studied and becomes progressively later as the dose is increased. With the maximum dose it was about 2 hours after injection.

Similarly the return to the initial level is earlier with the lighter doses. With a dose of U/16 the initial level is reached $2\frac{1}{2}$ hours after the injection,

with doses of U/8 and U/4 after 3½ hours. With the heavier dosage the initial level was not touched during the period of observation.

The shape of the dosage-sugar drop curve reveals a difficulty in differentiating the heavier dosages and indicates that insulin assay should be made with lighter doses.

The greatest precision for all dosages was at the time of maximal drop, during the period of early recovery the precision was appreciably lowered. Minor factors in determining the amount of drop are the initial sugar level and the total dosage.

The pancreas and the thyroid apparatus. FREDERICK S. HAMMETT.

The production of a thyroid deficiency (thyro-parathyroidectomy) in the male and female albino rat at different ages (23, 30, 50, 65, 75 and 100 days) is followed by a retardation of the growth in weight of the pancreas which increases in degree with the increase in the age of the animal at the time of the initiation of the glandular deficiency. The direction of the change in response of the organ with advancing development is like that shown by the body weight. The same similarity in pancreas and body weight response is exhibited in conditions of parathyroid deficiency occasioned at progressive ages. Since both thyroid and parathyroid deficiency are productive of a decrease in the amount of material available for growth it is clear that the growth processes of the pancreas are similar to those of the body as a whole and hence not unjquely related to thyroid or parathyroid activity as such.

The degree of retardation of pancreas growth is greater in the case of thyroid than in the case of parathyroid deficiency. Hence it is clear that the pancreas is more dependent upon the maintenance of a normal metabolic rate than upon the preservation of the nutritional level. This is supported by the fact that the degree of retardation of pancreas growth is practically the same as that of the body as a whole in conditions of parathyroid deficiency; while in conditions of thyroid deficiency the growth of this organ is retarded more than that of the body. This greater sensitivity of the pancreas to the lowered metabolic rate may be attributed to its inherent high normal functional activity and not to any specific thyroid relationship.

Of great significance is the fact that the growth processes of the pancreas in their relation to the metabolic and nutrient levels are strongly conditioned by the factors introduced at puberty—factors which presumably emanate from the surge in the incretory activity of the gonads occurring at this time. This is shown by the fact that the growth of the pancreas subsequent to thyroid removal is not only absolutely stopped at the beginning of puberty but is even retrogressve (the organ loses weight) and the fact that a marked increase in the degree of retardation occurs at the same time under conditions of parathyroid deficiency.

Additional observations on Eck-fistula thryoparathyroidectomized dogs. JULIUS BLUMENSTOCK, WILBUR R. TWEEDY and LONDUS BRANNON.

In an earlier communication[1] we stated that the symptoms following thyroparathyroidectomy in Eck-fistula dogs differ from those of ordinary animals. We now wish to record the following additional observations:

[1] Blumenstock and Ickstadt. Journ. Biol. Chem., 1924, lxi, 9.

1. Four animals in the series showed meat intoxication, and all went into tetany without the usual preliminary symptoms or signs, and died in spite of all treatment.

2. Such animals that do not exhibit meat intoxication before thyroparathyroidectomy may be controlled easily by the oral administration of calcium salts, and have in two instances passed into a state not necessitating further treatment. One animal (R) has survived more than a year, and to all appearances is a normal animal. The other (S) has survived over 8 months and is outwardly normal.

3. Lenticular cataracts which usually appear in dogs about the sixth month after parathyroidectomy,[2] and progress so that the vision of the animal becomes definitely impaired within a year, were slow to appear in the first animal (R). The first cataract was observed at the eleventh month, and at the end of the seventeenth month had progressed practically none.

4. A value of 11.5 mgm. of calcium per 100 cc. of plasma was found in the dog (S) seven months after the establishment of an Eck-fistula. After parathyroidectomy in three instances the plasma calcium has been found to fall as low as 3.58 to 3.70 mgm. after 48 hours' starvation, without any symptoms other than a transient shivering, although the animal was under no treatment whatever.

Such facts as the above are advanced as additional evidence that the syndrome following parathyroidectomy is markedly changed in Eck-fistula dogs, and moreover shows that factors other than the level of the plasma calcium are responsible for the symptoms following parathyroidectomy.

The onset of the pot-belly in the thyroidectomized sheep and goat. H. S. LIDDELL[3] (by invitation) and E. D. SIMPSON (by invitation).

One of the earliest signs of experimental athyroidism is the depression of neuro-muscular activity indicated by lethargy and muscular weakness. Since the onset of the typical cretin pot-belly appears at about the same time, a special study of the distended abdomen as an indicator of neuro-muscular function was undertaken. Four pairs of twin lambs and one pair of twin kids were selected for observation. Both members of each pair were of the same sex and one twin of each was thyroidectomized at one month of age. Weekly measurements were made of body weight, chest and abdominal girths. The ratios of chest to belly circumference were computed every week and the date noted on which the ratio in the cretin exceeded that found in the control. This point was taken to indicate the onset of the pot-belly in the thyroidectomized animal. The weekly percentage increment in body weight was calculated and the date determined on which the growth rate of the thyroidectomized animal was definitely retarded as compared with its normal twin. It was then possible to compare the time of onset of the pot-belly with the time of the occurrence of marked growth retardation. The results of the comparison are shown in the table. Daily observation of abdominal girth showed much greater variability in the normal animals. The subcutaneous administration of thyroxin to the cretin goat caused, after a latent period of three

[2] Luckhardt and Blumenstock. This Journal, 1923, lxiii, (proc.); Dragstedt, Phillips and Sudan. This Journal, 1924, lxix, 474.

[3] Fellow of National Research Council.

days, a pronounced decrease in belly girth coincident with an increase in spontaneous activity as recorded by the pedomenter. That the occurrence of the pot-belly is not due to muscular degeneration was demonstrated by excising small slips of pectoral muscle from two typical cretin sheep (and their twin controls) one eight months of age, the other five and one-half years old. Muscle specimens from both thyroidectomized animals were normal in structure.

TABLE 1

CRETIN	POT-BELLY NOTED	RETARDED GROWTH NOTED
Kid 1	Third week after thyroidectomy	Third week
Lamb 1	Second week	Third week
Lamb 2	Second week	Third week
Lamb 4	Third week	Second week
Lamb 6	(Doubtful case)	

Some observations concerning metabolic rate in turtles, Chrysemys marginata belli and Chelydra serpentina (Linn). FRANCIS M. BALDWIN.

By the method of indirect calorimetry, the rate of gaseous exchange in turtles was obtained, and some of the contributing factors analyzed. This report concerns experiments where the temperature was maintained within narrow limits (about 20°C.). The first series of experiments was carried out (for twenty-four-hour periods) upon seven large individuals of the painted turtle, each weighing approximately 1200 grams, after being kept in the laboratory four weeks without food but with water sufficient to keep the body moist. Oxygen consumption figured on the cubic centimeter gram hour basis showed very close agreement, within each of the series. The limits were from 0.0327 to 0.0207 cc. gram hour, with an average of 0.0261 cc. gram hour. Another series, from individuals of the same species of comparable weights, taken directly from the natural environment showed a range from 0.0453 to 0.0536 cc. gram hour, with an average of 0.0492 cc. gram hour. A series of experiments performed on snapping turtles taken directly from the water, and weighing about 1700 grams each, showed a range from 0.0431 to 0.0883 cc. gram hour, with an average of 0.0562 cc. gram hour. On the cubic centimeter kilo/day basis, the average figures in the three series become 604.8; 1,169.0; and 1,490.2 cc. kilo/day respectively. Assuming, 1 liter O_2 = 4.60 cal. @ 20°C., these figures are of interest when compared with figures for basal and total energy metabolism computed for man on the same basis. Taking 400 liters O_2 per day (1,840 cal.) as the average consumption in man under basal conditions, the ratio in the first series cited becomes approximately 800:1. When the animals, as in the second series, are taken directly from the water it is apparent that their metabolism is slightly more than doubled. Figures are not at hand as an index for the basal rate for the snapping turtle but it seems likely that the figures cited in the third series of experiments are relatively significant. This increment of energy expenditure would seem to be counterbalanced by the greater activity of the freshly caught animals. If the shell and hard parts of the body be deducted from the weight (roughly $\frac{1}{3}$) and the figures computed on O_2 consumption of soft parts alone the results compare favorably with figures obtained on the turtle by Rubner by the direct method.

Studies on Thiry-Vella fistula in dogs. SAMUEL AMBERG and FRANCES SAWYER.

Jejunal fistulas were established in three dogs by each end of the separated loop being sewn into the abdominal wall. The contents of the loop for various periods of time were obtained after a day's fasting and also after a meal of meat. After the meal the amount of secretion for each hour may increase or decrease in the different animals, and returns to the rate of the hunger secretion after about six hours.

The secretion is made up of flocculent and liquid parts. As a rule the greater percentage of the weight of the total secretion during hunger consists of flocculent material. The reaction determined directly at the mouth of the fistula with neutral red paper varied between a hydrogen ion concentration of 8.2 and one of 9.0. The content of each 100 grams of fluid secretion was as follows: sodium 315 mgm., potassium 35 mgm., calcium 7.4 mgm., magnesium 6.2 mgm., chlorin 267 mgm., and phosphorus 9 mgm.; whereas each 100 grams of the flakes contained sodium 321 mgm., potassium 35 mgm., calcium 27 mgm., magnesium 9.5 mgm., chlorin 376 mgm., and phosphorus 19 mgm. The ether-soluble residue of a desiccated turbid fluid was 0.23 per cent, that of a clear fluid 0.04 per cent. The greater amount of calcium in the flocculent material may readily be due to the alkaline reaction of the medium.

The rate of filtration of some aqueous protein solutions. SAMUEL AMBERG and FRANCES SAWYER.

With alundum crucibles set in a rubber ring and a pressure of 30 mm. of mercury, uniformity of experimental conditions is obtained sufficient to demonstrate conveniently marked differences in the rate of filtration. The water for the solutions is preferably redistilled in an all glass apparatus to avoid possible filter pollution. The experiments were carried out with solutions of gelatin, caseinogen, mucin, and ovomucoid.

Mucin in a dilution of 1:100,000 filtered more slowly than solutions of other proteins in dilutions of 1:1000. The reaction of the mucin solution exercises an influence, the slowest filtration occurring at about neutral reaction (pH 7.0). Acid solutions filter faster than alkaline. With a given protein solution the rate of filtration slows with increasing concentration. Neither concentration or viscosity nor surface tension are the sole determining factors with regard to the rate of filtration of different protein solutions. For instance, blood serum or blood plasma filters considerably faster than some 1:100,000 mucin solutions, in spite of their much greater concentration and viscosity.

The treatment of pleural effusions by the oral administration of thyroid or the intravenous injection of hypertonic saline solution. C. S. DANZER (by invitation).

In 1917 Eppinger[1] showed that certain cases of generalized subcutaneous edema, usually attributed to an insufficiency of the heart or kidneys, were associated with a disturbance in thyroid function. He arrived at this conclusion because of the therapeutic results following thyroid feeding. In such cases the anasarca, which had hitherto been refractory to the

[1] Eppinger, H. "Zur Pathologie Therapie des Menschlichen Odems." Berlin, 1917.

methods employed in the treatment of cardio-renal edemas, disappeared under thyroid therapy. In thyroidectomized animals the subcutaneous injection of normal saline remained as a localized edema instead of being absorbed as in the normal animal. The drinking of water by thyreoprivic animals likewise failed to produce the usual diuresis. It became apparent therefore that the thyroid regulates the transport of water to and from the tissues. In pathologically underfunctioning thyroids, perhaps too slight to produce the myxedema syndrome of hypothyroidism, this form of dropsy might occur.

It seemed to us that a similar explanation might apply to the pleural effusions which remained after the underlying cause had passed off, as in the case of pneumonia, or other obstinate cases of exudation into the pleural cavity. We preferred to regard these as pleural edemas.

We therefore subjected eight cases of pleural effusions with positive clinical signs and confirmed by x-ray or aspiration, to thyroid treatment. In five cases thyroxin was used, in the remaining three desiccated thyroid substance. The dose of the former varied from $\frac{1}{6}$ mgm. to $\frac{1}{2}$ mgm. repeated three to four times daily, in the case of the latter six to twelve centigrams were given at similar intervals. In most of the cases seven to eight days were allowed to elapse before treatment was begun in order to estimate the degree of spontaneous absorption.

The results in six out of the series were very striking. Distinct diminution in the amount of fluid was noted both physically and roentgenologically on the fourth day. On the eighth to the twelfth day most or all of the fluid, depending on the pre-existing amount, had disappeared. In none of the cases did any signs of hyperthyroidism develop unless massive doses of thyroid were given.

Inasmuch as our results were not good in two (25 per cent) of our series, we attempted to reduce the amount of fluid by another method, namely, the intravenous injection of hypertonic saline solution. We employed a 10 to 15 per cent solution and injected 20 cc. two times daily. We assumed that the osmotic effects might help to accomplish the desired end. In order to retain the absorptive effect by retarding the rate at which the sodium chloride might pass out of the circulation, the saline solution was mixed with the patient's blood drawn into the syringe before it was injected. This method, it was thought, might bring about a salt protein combination which might remain for a longer period in the vascular system, thus allowing for a prolongation of the dehydrating effect on the effusion in the pleura.

This method also proved very useful. Practically after the first day the subjective symptoms due to the fluid collection in the pleura, showed a marked amelioration. Thus the pain in the chest, the dyspnea, the cough, weakness, restlessness, insomnia and nausea practically disappeared. At the same time the urinary output exceeded by far the fluid intake as soon as this treatment was begun. A slight reduction in bodyweight occurred. The loss of weight was quite parallel with the excess in fluid output.

The two cases in which the combination of hypertonic saline and thyroid administration was tried had both been aspirated before and were refractory to the usual therapeutic methods. In one instance it was done three times, in another case once. The fluid in identical or greater amounts returned, one instance filling the entire chest on the affected side.

It is therefore suggested that pleural effusions that fail to be absorbed

spontaneously and which cause distressing or other symptoms demanding treatment, be given thyroxin or other active thyroid substance. Should these be insufficient, hypertonic saline solution may be injected intravenously twice daily until the fluid disappears or is sufficiently reduced to permit of the expectant plan of treatment. The most striking effect is to be seen from the simultaneous application of both methods.

Observations on the function of the gall bladder. L. R. WHITAKER and E. A. BOYDEN (by invitation).

A. The rhythmic nature of the biliary flow (Boyden and Whitaker). Cholecystograms of the human gall bladder taken at 15-minute intervals after a meal demonstrate that ingestion of egg yolk and cream empties a distended gall bladder in two and one-quarter hours. Meat is erratic in its behavior, usually causing a partial emptying of the gall bladder within three to four hours, but in one instance complete collapse was induced in four and one-half hours. As will be pointed out below, contraction begins at least five minutes after eating.

Examination of successive cholecystograms from a given subject suggests that the gall bladder does not empty continuously but periodically. Thus with a diet of yolk and cream the curve of contraction, obtained by plotting successive x-rays of the gall bladder, shows three discontinuous impulses. With a diet of meat, in which partial emptying is soon followed by refilling, a similar periodicity is exhibited in filling as well as in emptying. This observation suggests that we are not dealing with an intrinsic rhythm of the gall bladder so much as with a periodic action of the sphincter papillae. This view is strengthened by two instances in which the gall bladder increased in size between successive contraction phases, indicating an inflow of hepatic bile and a closure of the duodenal sphincter.

In testing this periodic contraction of the gall bladder in experimental animals, a supplementary rhythm, not dependent on the sphincter, was discovered in the flow of the hepatic bile. When the sphincter was eliminated by inserting a cannula into the common bile duct of a chloralosed cat and the hepatic bile suddenly released into a glass tube (the cystic duct being clamped), the flow of bile from the common duct exhibited conspicuous rhythm with slowly diminishing intensity, the successive wave lengths occurring at approximately ten-minute intervals over a period of several hours. In several instances the rhythm was revived by the injection of pituitrin. It is believed that this represents a rhythm of the musculature of the biliary duct system (rather than of the secretory mechanism of the liver) and is the means by which the bile is milked down the hepatic ducts into the duodenum.

B. The mechanism for emptying the gall bladder (Whitaker and Boyden). In experimental animals it is possible to demonstrate the exact time at which bile first leaves the gall bladder. This has been accomplished by injecting iodized oil into the fundus of the gall bladder and then feeding these animals with egg yolk as soon as they have completely recovered from the operation. X-rays taken at frequent intervals show that iodized oil enters the cystic duct within five minutes after feeding; in ten minutes the oil outlines the course of the common bile duct; and in fifteen minutes after feeding it appears throughout the small intestine. This observation proves the truth of the historical conception that the primary function of the gall bladder is to deliver bile to the intestine following the ingestion of appropriate food.

What is the force which expels bile from the gall bladder? Is it primarily some external pressure or the contractile force of the gall bladder musculature? By means of the procedure outlined above we have been able to demonstrate that the muscle is an effective agent. When pituitrin or barium chloride is injected intravenously into cats in which the gall bladder is filled with iodized oil, passage of oil into the cystic duct can be demonstrated by x-rays within three minutes. Further experimentation shows that the emptying of the gall bladder is not due primarily to reflex action since denervation of the organ does not inhibit its emptying after the ingestion of egg yolk. Complete emptying depends upon the opening of the sphincter of Oddi, for without this action the gall bladder does not discharge its contents. Conversely, when the sphincter papillae is eliminated by an inlying cannula, the gall bladder does not fill. Two essential factors in this mechanism would therefore seem to be the intrinsic contractile power of the gall bladder and the relaxation of the sphincter of Oddi in response to food.

The effect of insulin on the rate of disappearance of reducing substances in toad's blood at different temperatures after injection of glucose. J. M. D. OLMSTED.

Toads injected with insulin show convulsions in approximately 36 hours at 18°C., 24 hours at 25°C., 18 hours at 30°C. At 18°C. the reducing power of the blood increases to a maximum of approximately 0.3 mgm. per cent 12 hours after injection of a standard dose of glucose; 24 hours after injection of glucose the reducing power is 0.05 mgm. per cent. If insulin and glucose are injected together at 18°C. the reducing power of the blood reaches the maximum in 6 hours, and after 18 hours falls to 0.03 mgm. per cent. At 25° the reducing power reaches a maximum within 1 hour after injection of glucose and after 12 hours has fallen to 0.04 mgm. per cent. If insulin is allowed to act in the body for 21 hours at 25°, then glucose injected, the reducing power of the blood is lowered to 0.06 mgm. per cent in 3 hours. If the insulin is allowed to act in the body for less than 21 hours then 3 hours after injection of glucose the reducing power of the blood is always high, i.e., 0.2 mgm. per cent. These results can be explained on the assumption that insulin reacts on substances in the body, a reaction rather slow and dependent upon the temperature, to produce an intermediary substance, and it is this intermediary substance which acts on the reducing substances of the blood and is responsible for convulsions.

A study of the effect of moderate doses of alcohol on the growth and behavior of the rat. CURT P. RICHTER.

This work forms part of a series of experiments undertaken primarily for the purpose of investigating the factors involved in the production and modification of activity in animals and man. The starting point for the present work was to determine the extent to which alcohol modifies spontaneous activity in the rat. In the course of the development of the experiment several unexpected observations were made on the more metabolic aspects of the problem and these were followed up intensively. The results of these observations now form an important part of the work.

1. It was found that the white rat is able to utilize an 8 to 16 per cent solution of alcohol as its steady fluid supply, replacing isodynamic quanti-

ties of food without intoxication or habituation effects, but with a definite decrease in spontaneous activity.

2. It was shown that the ability of the rat to digest such large quantities of alcohol without serious effects was due to its high rate of metabolism. The amount of alcohol taken by the rats was 5.1 to 7.8 grams per kilogram body-weight. The same amount for a man weighing 70 kilos would mean a daily intake of 357 to 546 grams or 0.450 to 0.688 liter, which is above what can be taken without intoxication effects. In Atwater and Benedict's experiments 1.03 grams per kilogram body-weight were given, which is $\frac{1}{5}$ to $\frac{1}{7}$ as much as the rats receive. When, however, the difference in energy requirements between rats and man is taken into account, the doses taken by the rats and those given to man in Atwater and Benedict's experiments turn out to be the same. This may be shown in another way by comparing the proportion of the energy of the alcohol to the total energy-intake in rats and man. The rats took 1.52 to 2.28 grams, 10.81 to 16.24 calories, or 22.0 to 28.8 per cent of the total energy-intake. Atwater and Benedict gave 72 grams per day; that is, 511 calories or 23.1 per cent of the total energy-intake.

3. It was found in these experiments, carried on over a long period of time, that rats which took alcohol ate 16.9 to 35.6 per cent less than controls which drank water. In spite of the fact that they ate so much less they grew just as rapidly and reached the same body-weight at maturity as the controls. The energy of the alcohol, calculated in calories per kilogram body-weight, exactly balances the energy of the difference in food-intake between the alcohol and control animals. The conclusion was drawn that in the rat alcohol not alone replaces isodynamic quantities of food in maintaining energy balance, as was demonstrated in man by Atwater and Benedict, but also that it is used for growth and development.

Intestinal extract (secretin) as a hematopoietic stimulus. JOSEPH T. KING (introduced by F. H. SCOTT).

It has been claimed that extracts of the intestine (secretin) have a stimulating effect on blood-forming tissue.[1] The subcutaneous injection of intestinal extracts into rabbits does produce an increase of red cell count. This increase, however, is probably not due to an increased production of cells, but due to a loss of plasma. The blood after injection shows no signs of excess regeneration. Histamine and peptone products produce similar results.

Long-continued injections (30 to 40 doses in 8 weeks) have no real effect on the blood. The blood of the control animals fed the same and kept under similar conditions is practically identical with that of the treated animals.

The "effective" osmotic pressure of the plasma proteins. G. F. FAHR and W. W. SWANSON.

The "effective" osmotic pressure of the plasma proteins is the osmotic pressure actually exerted by these proteins minus the counter pressure due to the Donnan equilibrium. The "effective" osmotic pressure of the plasma proteins measures the water-holding power of the plasma. The blood pressure in the capillaries must be greater than the "effective" osmotic pressure if filtration is to proceed.

[1] Downs and Eddy. This Journal, 1918, xlvi, 209.

The fluid on the outside of an osmometer should correspond as nearly as possible to the filtrate outside the capillaries in order that measurements of the "effective" osmotic pressure be accurate because the Donnan equilibrium counter pressure depends upon the composition of the outer fluid. As outer fluid for our measurements we used anasarca fluid obtained on one occasion from the sub-cutaneous tissues of a case of subacute glomerulonephritis and on another occasion from a case of complete obstruction of the inferior vena cava with marked anasarca of the lower extremities. This fluid flowed freely through Southey tubes under a pressure of 10 cm. of water. The albumin content of the first fluid was 0.08 per cent, there was no globulin present. The second fluid obtained contained 0.07 per cent albumin and no globulin. There was about 0.012 per cent fibrin in both fluids. These fluids oxalated were used as outer fluids for the osmometer which was a collodion sack holding about 20 cc., and attached to a mercury manometer. The oxalated pooled blood plasma from three patients in the hospital was used for the first measurement and the oxalated blood plasma from two other patients used for the second measurement. These patients were not suffering from nephritis nor did they exhibit signs of edema. Their water excretion was normal and the plasma proteins about 5 per cent albumin and 2 per cent globulin.

The collodion sacks were not permeable to serum albumin and globulin. The error of the measurements was not greater than $\pm$ 10 per cent.

The first measurement gave a value of 21 mm. Hg for the "effective" osmotic pressure of the plasma proteins at 37°C. The second measurement gave a value of 22 mm. Hg for the "effective" osmotic pressure of the plasma. We believe that these measurements are very close to the actual "effective" osmotic pressures of normal human plasma as it exists in the capillaries of the lower extremities. The values are about 10 mm. Hg lower than those found by Starling (Journal of Physiology, Vol. 24, 1899, 317). Starling assumed that the relation between the osmotic pressure of the serum proteins and the percentage concentration of these proteins was a linear relation. Schade and Claussen have shown that the osmotic pressure increases more rapidly than the concentration of the serum proteins. Starling used serum protein concentrations of 11 per cent. If we extirpolate Starling's values obtained with an 11 per cent serum protein concentration for a concentration of 7½ per cent making use of Schade and Claussen's curve, we obtain a value of 23 to 24 mm. Schade and Claussen (Zeitschr. f. klin. Med., c, 1924, 363) measured the osmotic pressure of the plasma proteins of ten patients with no nephritis or edema and with normal plasma protein content. Their mean value was 25 mm. Hg at 37°C. the lowest value was 21 mm. Hg and the highest 28 mm. Hg. Our value is about 3 mm. Hg lower than their value. This difference can be explained by the assumption that in Schade and Claussen's method some pressure is necessary to push the protein-free plasma through the membrane in addition to the pressure necessary to compensate for the osmotic pressure of the proteins. Schade and Claussen made use of a chamber closed above by a collodion membrane and filled with citrated plasma. Mercury was placed upon the membrane and the protein-free plasma slowly pushed through the membrane. By observing the pressures exerted and the volumes moved the "effective" osmotic pressure can be calculated. In the method of Schade and Claussen the fluid on the outside of the manometer is eliminated. We are not sure

that the Donnan equilibrium under these circumstances is reached, or that the Donnan counter pressure is the same as that actually existing between the plasma in the capillaries and the fluid outside. An objection to our measurement is the fact that the anasarca fluid may not represent the ultrafiltrate actually found outside the capillaries. We believe it is the nearest approach to such a fluid. (Carried out with the aid of a grant from the Ella Sachs Plotz Foundation.)

The refractory phase in relation to the action potential of nerve. J. ERLANGER, G. H. BISHOP and H. S. GASSER.

One of the proofs of the composite nature of the action potential wave of mixed nerve[1] is the relation the absolutely refractory phase bears to the action potential wave. This relation, which is readily determined by means of the cathode-ray oscillograph, is here summarized. The usual method of determining the duration of the absolutely refractory phase, namely, recording at one end of the nerve the action potential resulting from double stimulation of the other end, gives values (to cite two examples) of 1.20, 1.68 and 3.13σ for the fastest of the alpha, beta and gamma fibers, respectively, of the sciatic of the green frog, and 1.42, 2.06 and 4.46σ in the bullfrog (room temperature). Owing to the increase in the length of the action potential wave as it moves along the nerve comparison of the durations of action potential and refractory phase must be made at the site of stimulation. Since, however, the alpha, beta and gamma waves are all presumably of the same duration there[2] it follows that there is no fixed relation between the durations of these phenomena. It may, therefore, be merely a coincidence that in the case of α the durations are nearly alike at the site of stimulation.

To ascertain the relation of the refractory phase to the propagated potential wave the second stimulus is applied at some point along the course of the nerve, and a record is made of the position of the escape of this stimulus in the moving potential wave where the former just fails to start a second process. It is thus found that the refractory phase of, say, the alpha fibres ends the same interval after the beginning of the wave irrespective of the increase in the duration of the latter as it moves along the nerve.

By means of somewhat similar methods it can be shown that the immediate entrance of a nerve into the refractory state with the beginning of the action wave holds only when the two stimuli are applied to the same point on the nerve. When the second stimulus is placed in the beginning of the propagated action wave it can be shown by a resulting change in the form of the action wave beyond, that the nerve does not become refractory immediately with the development of the potential. This initial unrefractory period varies with the distance the wave has moved along the nerve.

This shift of the beginning of the refractory phase with respect to the beginning of the moving potential wave and of the end of the refractory phase with respect to the end of the moving wave are necessary consequences, indeed constitute one of the proofs, of differences in the rate with which individual fibers conduct their impulses.

[1] Erlanger, J., G. H. Bishop and H. S. Gasser. This Journal, 1925, lxxii, 197.
[2] Erlanger, J., H. S. Gasser and G. H. Bishop. This Journal, 1924, lxx, 624.

Respiration in fowls. C. C. GUTHRIE.

This work is part of a study of respiration in asphyxia. It comprised two major considerations: rate of oxygen consumption and oxygen capacity in ducks and chickens. Several related questions were studied.

In relation to body weight, oxygen consumption and carbon dioxide elimination were about the same, when by ventilation the carbon dioxide content of the inspired air was kept under 3 per cent, though the surface area was about 25 per cent greater in the ducks. The difference in feathering may be a factor. In a few experiments, rebreathing without ventilation with the carbon dioxide rising to 7.5 to 12 per cent, ducks absorbed 20 per cent the most oxygen.

Blood volume was about 50 per cent, blood oxygen capacity 20 per cent and vital capacity 100 per cent greater than in chickens.

Calculation of maximal oxygen capacity (blood and air), gives ducks almost 100 per cent more (per kilo) than chickens, and approximately agrees with the difference in their resistance to asphyxia, ducks surviving two or three times the longer (Bert; Richet).

Observations on inherent cellular resistance to asphyxia as toleration of anesthetics and of hemorrhage with injection of salt solution, and persistence of reflex and convulsive movements after decapitation, suggest greater resistance in the duck.

A great difference was observed in reaction to chloroform. Administered in lethal dosage, often chickens are dead before ducks begin inhalation, the former "holding their breath" for only ten to thirty seconds, and the latter up to about four minutes.

Studied by placing in a closed chamber, no striking differences in reaction to such asphyxiation, to a point where the air oxygen and carbon dioxide contents approached 9 to 10 per cent, were observed.

Inhibitory reactions described by others as occurring in submerged ducks (Richet; Huxley; Paton) have not been observed.

Heart and lung weights in ducks in relation to body weight were nearly 100 per cent greater. The arteries and veins are correspondingly large.

The fowls had free access to food and water until shortly before being studied. Fifteen of each species have been used and each comprised several varieties. Also both sexes, various ages and different nutritional states were represented. In agreement with others, individual variations of considerable magnitude were noted. This summary is based on averages.

The record of the action potential of nerve at the site of stimulation. G. H. BISHOP, J. ERLANGER and H. S. GASSER.

Using the cathode ray oscillograph and amplifier, the record of the action potential of nerve close to or at the site of stimulation shows a large "escape" distortion which overlaps the potential wave itself. This distortion is due to polarization of the nerve by the stimulus and the electrotonic currents resulting from it, and to capacity in the stimulating circuit. It can be virtually eliminated by placing the nerve in one arm of a wheatstone bridge, such that the cathodal stimulating region and an opposite point of the bridge are at the same potential whatever stimulating current is passed. This opposite point is grounded, and serves as the ground lead for the nerve action potential, through the bridge, the grid being on the killed end as usual. The oscillograph is then a null-point detector of the bridge balance as well as a recorder for the action potential.

With this circuit, using a galvanic current to stimulate, we may observe the excitation response with the record of stimulation eliminated. The action wave rises under the cathode from the zero of the polarization produced by the stimulus (which is balanced out) with essentially the same shape and size as elsewhere. At all current strengths that stimulate, the excitation is observed to start without lag as the adequate stimulation is accomplished, disappearing as the end of current duration is shortened back to the exact start of the wave. When strong stimulating currents are allowed to continue flowing during the response, the action wave is depressed due to cathodal polarization by the stimulating current, before it has time to rise to its maximum, although it does rise to maximum away from the cathode. Shortening the duration of stimulation nearly to the start of the wave removes this polarization depression, and allows the maximum to be again attained at site of stimulation. The wave may then even increase in amplitude here with increase of current after a maximum has been reached at the other points of the nerve.

By the above technique we may also study the process of stimulation separated from the response. During the utilization period of a just threshold galvanic stimulus, induction shocks are applied at the cathode which elicit threshold responses earlier, immediately following the shocks. The strengths of these shocks measure the extra stimulation that must be applied to cause responses at their respective times. Subtracted from the threshold shock strength without the galvanic stimulus, they measure the stimulating effect which the galvanic current flow has accomplished after any given time. Plotted against time, these latter values fall not upon a straight line, but upon a decremental curve, such that any given increment of duration of constant stimulating potential is seen to have a greater stimulating value than the succeeding increment.

Many other similar properties of nerve have been investigated by modifications of the above circuit.

Blood sugar and urinary D:N ratio in the hours following pancreatectomy. WILLIAM H. CHAMBERS.

The daily excretion of sugar and nitrogen in depancreatized dogs is well known, but no records have been found of the nitrogen excretion or dextrose:nitrogen ratio at frequent intervals during the first 24 hours after the operation. In the following experiments on female dogs which have fasted for 48 hours the urine determinations have been made in hourly periods for the first 9 hours after pancreatectomy, and then in 3-hour periods until the 24th hour. By using amytal for the anesthetic it is possible to follow the changes in blood sugar coincident with the urinary excretion.

During the operation there is a distinct fall in the nitrogen excretion and no significant change in the blood sugar concentration. A rapid rise in the urinary nitrogen and the blood sugar follows the pancreatectomy. The increase in total nitrogen per hour amounts to between 100 and 200 mgm. in the first 6 hours, as compared to a nitrogen increase of 100 and 140 mgm. during the same period in the control laparotomy and partial pancreatectomy. There is little variation in the rise of the blood sugar curves. In two dogs the renal threshold is reached at 0.150 per cent in about 6 hours, while in the other two the sugar did not appear in the urine until the twelfth hour at blood sugar levels of 0.195 and 0.240 per cent.

A continued rise in the nitrogen and sugar excretion is found until both reach a maximum at about the twenty-first hour. The elimination of the glucose is the more rapid at first; thus the highest D:N ratio occurs about the fifteenth to eighteenth hour.

The rise in the blood sugar level immediately after pancreatectomy[1] when compared with a constant level in the control laparotomy indicates an immediate effect of the lack of insulin. The first marked effect on the nitrogen excretion is noted between the sixth and twelfth hours, and by the twenty-fourth hour two cases have reached the nitrogen level of a constant protein metabolism and the constant diabetic D:N ratio of 2.8.

Studies in the nutrition of the white mouse. HOWARD H. BEARD.

This investigation was undertaken to study in considerable detail some of the important problems in the nutrition of white mice, with emphasis upon the rôle of the vitamins in this connection.

The results obtained were as follows:

1. When fed upon the standard casein diet of Osborne and Mendel, with a level of 31 per cent casein, the females attained a maximum weight of 27 grams, and the males a maximum weight of 34 grams.
2. Normal growth was observed when a low casein diet was supplemented by a small amount of cystine.
3. Taurine could not be substituted for cystine in the diet of mice.
4. No hypertrophy of the kidneys was observed when the mice were fed on diets containing from 75 to 80 per cent of casein.
5. Vitamin A proved to be necessary for growth, but the absence of it did not produce xerophthalmia.
6. Vitamin B also proved necessary for growth, 40 mgm. of yeast vitamin powder (Harris) being the lowest daily amount that would give maximum growth up to 18 grams body weight. The requirement of the mouse for this vitamin, like that of the rat and the dog, was found to be in linear relationship to the product of a, its weight, b, its surface, and c, the number of calories metabolized per day.
7. Vitamin C proved unessential for normal growth, and no scorbutic symptoms were observed in the case of diets free from this factor.
8. Mice fed upon diets supposedly free from calcium or phosphorus did not develop rickets.
9. Vitamin E was found to be necessary for the normal reproduction of the mouse, reproduction being impossible upon the standard casein diet. Lettuce completely cured the sterility.

The resting metabolism of infant rats in relation to temperature control. ADDISON GULICK.

Well-conditioned and well-nourished rats in the first few days of life produce from 7 to 9 calories per kilogram, the rate staying the same at 24° and 16° as at 31°C. But in starved rats (2 days old), metabolism at 20° is less than half of the 30° rate. Shortly before 11 days the rats develop shivering and other muscle reflexes that greatly augment the gas exchange between 18° and 26°C., but the basal (warm chamber) rate per kilogram continues as before. The suggestion is offered that this basal metabolism is close to the physiological limit of heat production in a relaxed organism.

[1] Hédon, E. and G. Giraud. Compt. rend. Soc. Biol., 1920, lxxxiii, 330.

Adrenalin and the tonus of decerebrate rigidity. EUGENE L. PORTER.

It has been contended that sympathetic innervation is responsible for a part of the tonus of decerebrate rigidity. If this sympathetic innervation be of the typical form adrenalin injected into the blood should cause contraction. Attempts to bring this about have failed.[1]

Gruber,[2] on the other hand, injecting massive doses of adrenalin intravenously found invariably an improvement in the contraction height of a muscle if the injections were given while it was being stimulated regularly by induction shocks. The improvement was greatest in muscles which had been fatigued by long-continued stimulation. It therefore might be conjectured that adrenalin would increase the contraction height of a muscle in decerebrate rigidity, particularly if a part of that rigidity be caused by sympathetic innervation. Especially one might look for the effect in a muscle whose rigidity was diminishing, since a part of the diminution would presumably be due to peripheral fatigue. Gruber contends that adrenalin lowers the threshold of the muscle to stimulation. One might imagine that in a muscle showing a slowly decreasing rigidity, fibers which had just ceased to function might be thrown back into action by adrenalin. I have based my procedures in the present investigation on this idea. Cats decerebrated by Sherrington's guillotine method were employed. The muscle used has been a small extensor of the tail, one of the many grouped under the term of extensor caudae lateralis. The animal rests in a cradle hollowed out of a block of wood to conform to the shape of the body, head supported, legs and tail hanging free. The muscle employed has its origin on or near the last of the lumbar vertebrae. The spinal column at this point is immobilized by a specially designed clamp shaped like a two-pronged fork. The prongs can be approximated by screws. The clamp is applied on the opposite side of the spinal column from the muscle, one half gripping the ventral side of the spinal column along the centre of the vertebrae, the other the dorsal alongside the spinous processes. The two parts of the clamp are screwed together after being placed in position and the shank of the clamp made fast to the wooden block forming the cradle for the animal. The tendon of the desired muscle is isolated and attached to a lever for graphic registration. Increased contraction of the muscle, i.e., extension of the tail is usually brought about by rubbing the fur of the tail the wrong way. As the contraction dies down adrenalin hydrochloride (usually 1 cc. of a 1:10,000 solution in saline) is injected into the external jugular vein. In no case has an effect on the existing contraction height been observed. The muscle continues to relax at the same rate as when no adrenalin has been administered. If experiment is varied by giving the injection just before the production of the increased rigidity, or while the stimulus to contraction, as rubbing the tail, is actually going on, no change in the character of the subsidence of the contraction has been detectable. Rate and smoothness remain the same.

The results of this investigation are therefore against the view that the tonus of decerebrate rigidity is due to sympathetic innervation.

Some effects of parathyroid extract (Collip) on heart function. D. J. EDWARDS and IRVINE PAGE.

It has been shown that the administration of calcium by mouth or intravenously produces a rise in the calcium content of the blood but this

[1] Gayda T. Atti del. Reale Accademia Nazionale dei Lincei 33, 310, 1924.
[2] Gruber. This Journal, 1922, lxi, 475.

increase does not appear to be sustained for more than a few hours. The parathyroid hormone of Collip, on the other hand, causes a hypercalcemia which can be maintained and for this reason it offers a more physiological approach to the study of the influence of increased blood calcium upon the heart function in the intact animal.

Repeated injections of the parathyroid extract of Eli Lilly and Company have been given healthy dogs over an average period of 51 hours. The average dose used was 21.9 units per kilogram body weight. There was present commonly increased muscular tremor within 20 hours following the first injection, but later on, some degree of atony, nausea, and increased salivation were shown.

The heart rate shows a decrease during the first 24 hours under the parathyroid treatment and in this period a marked arrhythmia is commonly present. The frequent occurrence of nodal and ventricular extrasystoles suggests that there is some depression of the sinus node. At a later stage the rhythm becomes regular again and the rate much increased.

The blood pressure recorded from the femoral artery ranged from 48 to 90 mm. Hg. These measurements were made on animals that had been under treatment for from 29 to 72 hours and showed definite atony previous to giving the anesthetic. The pressure pulse, recorded with optical manometer, presents the characteristics of a high resistance curve even in instances where the mean pressure was below 90 mm. Hg. The systolic time is shortened.

The electrocardiographic records show no disturbances of conduction between auricle and ventricle (P-R interval), or within the ventricles (Q, R, S group). Marked alterations in the T wave occur; the most common being, inversion of this wave and a varying degree of potential between the R and T waves. The inconstancy of these variations, however, throws doubt upon their significance.

In terminal asphyxia the heart of these dogs with parathyroid hypercalcemia is peculiarly resistant and we have not observed it to undergo the acute dilatation usually exhibited under these circumstances. Moreover, animals dying from lethal dosage with the extract, show at autopsy marked engorgement and dilatation of the right auricle and ventricle and a firm tonic contraction of the left ventricle. There appears to be a definite cardiotonic effect from the parathyroid hypercalcemia.

Reflex and other effects on the empty stomach of the monkey. T. L. PATTERSON.

Acute experiments were carried out on Macacus and Ringtail monkeys under light ether anaesthesia to determine the effects of stimulation of certain sensory and motor nerve fibers on the empty stomach. The vagi, carotids and the esophagus were exposed in the neck. A lateral incision was made in the side of the esophagus for the introduction of a rubber balloon which was connected with a water manometer for recording the gastric movements and taken simultaneously with a blood pressure curve from the carotid or the femoral artery. Stimulation of the peripheral end of either vagus leads to a marked contraction of the stomach followed by a temporary inhibition in which there is a fall in tonus along with a sudden fall in blood pressure. After unilateral vagotomy, stimulation of the central end of the cut vagus usually leads to a reflex inhibition of the stomach with a fall in tonus and an increase in blood pressure.

The results of similar stimulation after double vagotomy are negative since the operation probably renders the stomach permanently hypotonic. Stimulation of the central end of a cut sciatic with the vagi and splanchnics intact usually leads to a temporary gastric inhibition, but in some cases there is an increase in gastric tonus. There is some indication that the sciatic may produce reflex inhibition of the stomach when in a state of hypertonic activity, or reflex augmentation of gastric tonus when in a state of rest. No effect is produced on the stomach from stimulating the peripheral end of the splanchnic although there is the usual increase in the blood pressure.

Effect of menotoxin injections on behavior of rats in the maze. D. I. MACHT and O. HYNDMAN.

Macht and Lubin[1] have described their studies on menotoxin first in these Proceedings and later in a fuller communication elsewhere.[2] It was shown that in the blood, sweat, saliva, milk and other secretions of menstruating women there is present a toxin, which is especially deleterious to plant protoplasm, but is also to a lesser degree toxic for animals and animal tissues. The chemical nature of this toxin was found by them to bear a relationship to oxycholesterin; and allied bodies such as cholic acid.

It is well known that at the time of catamenia, the female organism undergoes profound metabolic and other physiological changes. Very common concomitants of menstruation are pain, malaise, nervous irritability and psychic disturbances. In the present investigation an inquiry was made as to whether such symptoms may not be referred to the presence of menstrual toxins. Albino rats were trained to run in the circular maze, so as to perform that exercise in the shortest period of time and without errors. The rats were then injected with normal human blood serum on the one hand, and with blood serum from menstruating women, on the other hand, and the effect of the injections was observed. In order to avoid anaphylactic phenomena the injections were made in different rats, and when the same rats were used again, it was only after a long period of rest. Fifteen experiments were made with injections of normal human serum, and twenty-one with menstrual serum, the doses injected ranging from 0.01 cc. to 0.2 cc.

It was found that injections of normal serum produced no effect, or occasionally a slight transient depression. Injections of menstrual serum produced very marked depression of the animals, as manifested by their speed of running, loss of orientation, and numerous errors and frequently there was a distinct paresis of the hind legs. Recovery almost invariably followed in a day or two. Injections with alcoholic extracts of normal blood serum (evaporated and taken up in saline) and of menstrual serum gave exactly similar results as above. The results obtained in the rats were somewhat analogous to those obtained by the authors with cholic acid and cholesterin derivatives which are closely related to menstrual toxin.[3] Complete data to appear in the Journal of Comparative Psychology.

[1] Macht, D. I. and D. Lubin. Proc. Soc. Exper. Biol. and Med., 1923, xx, 265.
[2] Macht, D. I. and D. Lubin. Journ. Pharm. Exper. Therap., 1924, xixi,. 413
[3] Macht, D. I. and O. Hyndman. Journ. Pharm. Exper. Therap., 1924, xxii, 467.

Penetration of ultra-violet rays through animal tissues. D. I. MACHT, F. K. BELL and C. F. ELVERS.

Studies on the penetration of ultraviolet rays for tissues were made on living animals with the quartz spectroscope and spectrograph. For this purpose rabbits, cats and dogs were used. The animals were anesthetised, the skin of the abdomen was cut open and dissected back on the one side and then the barrel of the spectrograph was introduced under the skin. The skin was then radiated from the outside with Krohmayer and Alpine Sun Mercury Vapor Lamps and a spectrophotograph of the waves which passed through the skin was thus obtained while the animal was alive and the blood circulating. In this way it was found that a considerable number of invisible ultraviolet rays passed through the rabbits', cats' and dogs' skins. Thus in the case of rabbit skins varying from 1 to 2 or more millimeters in thickness, the spectrograph showed the lines in the region of 2800 angstrom units and sometimes even shorter wave lengths. In the case of the rabbit the longer ultraviolet rays penetrated not only through the skin but even through the whole thickness of the abdominal wall as shown by spectrographs made by the barrel of the instrument inserted into the peritoneal cavity. Here wave lengths of 3000 angstrom units were frequently obtained with both Alpine Sun and Kromayer lamps, although the thickness of the abdominal wall (skin, fascia, muscle and peritoneum) was usually about 3 to 4 millimeters.

The permeability of dead tissue was different from living animal tissue depending upon the state of preservation. Thus when skin was left at room temperature and began to undergo putrefaction the permeability was greater than in a normal living skin. On the other hand when skin was preserved either by freezing or by preservatives such as formalin or alcohol, the coagulation of proteins and other chemical changes thus produced rendered it more opaque. Leather for the same reason was found to be much more opaque than living skin. Human skin was studied by obtaining fresh specimens from the operating room and it was found that when not excessively thick it also allowed the penetration through it of the longer ultraviolet waves. A marked difference was noted between white human skin and the skin of the negro. In the latter case absorption of the entire ultraviolet region was noted. In rabbits and other animals under anesthetic, injections intravenously into the living circulation of fluorescent substances, such as eosin, produced complete absorption of the ultraviolet rays. The present investigation, which was begun in the fall of 1923, reveals conclusively that the penetration of ultra violet rays from modern powerful quartz lamps through animal tissues is much deeper than has hitherto been supposed. It is purposed to publish complete data in the near future.

The fate of depancreatized dogs kept alive with insulin. I. L. CHAIKOFF, J. J. R. MACLEOD, W. W. SIMPSON and J. MARKOWITZ.

On a diet consisting of 200 grams chopped lean meat, 50 grams raw pancreas, 50 grams sucrose, plus 16 units insulin twice daily, it has been possible to keep completely depancreatized dogs alive as long as desired. Two such animals are now alive two years after complete pancreatectomy. The post-absorptive blood-sugar in each is about 0.5 per cent. One of the animals went through a normal pregnancy and puerperium about one year ago, with no change in the sugar balance during the entire

course of the pregnancy. During the puerperium profound hypoglycemia developed. The animal suckled two pups. The urine, which previously had contained about 40 grams glucose per day, became practically sugar-free in the course of two weeks. These symptoms were ascribed to withdrawal of glucose from the maternal blood stream by the mammary gland for the manufacture of lactose.

The fact that these animals do not show the usual diabetic degenerations indicates that hyperglycemia *per se* cannot be their cause.

Withdrawal of insulin from these animals is always followed by pronounced glycosuria, reappearance of ketonuria, dehydration, and vomiting. There is no evidence that ability to metabolize glucose normally has reappeared in the two years that have elapsed since pancreatectomy.

Studies on spinal cord regeneration in the rat. R. W. GERARD and T. KOPPANYI.

One of us (T. K.) has demonstrated cord regeneration in amphibia and fish and desired to extend the work to mammals. The other (R. W. G.), from the clinical side undertook this study as a preliminary step in an attempt to secure return of function in adult human cord lesions by transplantation of embryonic cord. From this viewpoint, the question of whether function returns because of regeneration of injured cells or continued normal growth is not germane, though histologic studies in progress may decide this point.

Experiments were made on embryos in utero, recently born rats, and young adult rats.

Embryos were operated one to eight days before birth, average five, and from one to the entire litter were operated. The transparent uterus was exposed by a median incision and a strong transilluminating light rendered accurate observation possible. A cataract knife was stabbed through the uterus, the embryo steadied by slight pressure on the uterus, its spinal column in the upper thoracic region cut, and the knife withdrawn with very slight bleeding from the fetus and almost no leakage of amniotic fluid. In some, ink was injected into the leg for later identification. That the cords were actually cut was proven by removing, at operation, a number of operated embryos and dissecting them after formalin fixation. Further evidence may be available from microscopic study.

No abortions occurred unless the fetuses were within two days of term, maternal mortality was almost nil, and the infants were given care after birth. The embryos show remarkable healing powers. As soon as twenty-four hours after operation, it may be impossible to tell from examining the skin that anything has been done; and as severe a procedure as section of the spinal column, and probably even the aorta, can be survived.

Of seventy embryos, fifty-three were operated and many removed for study. Forty-eight embryos were allowed to develop, twenty-eight of these, or 58 per cent, having been operated. If the unfavorable assumption is made that all unoperated embryos were born alive and all those lost had been operated, which assumption is surely incorrect, 32 per cent of those operated were born alive, 21.5 per cent were found dead but fully developed and had survived until just before or after birth, 21.5 per cent were found dead and macerated, and had died in utero sometime before birth, 25 per cent were not found and had been eaten after birth or resorbed in utera.

The nine operated rats born alive showed voluntary and completely coördinated movements of the hind legs either at birth or one day after and six showed pain sensibility from the hind legs and tail at birth.

New-born rats were operated between three and fourteen days after birth, average five. No asespis was used, the spinal column being severed by one snip of iridectomy scissors or a stab with a cataract knife. Cuts were made at the high thoracic, low thoracic, and midlumbar levels.

Of thirty-six operated, twenty-five, or 70 per cent, survived till killed, those dying from the operation effects averaged three days life.

Twenty-seven were completely paralyzed and anesthetic below the lesion for at least seven days; nine showed partial function within this time and are considered as having been incompletely cut.

Three rats showed partial or complete motor recovery between seven and twenty-one days, and three showed both motor and sensory improvement. Of these only one had been entirely paralyzed and anesthetic from the start; it regained fair sensation and voluntary coördinated motion in both legs between fourteen and twenty-one days.

The high transection rats from the moment of section showed active stepping reflexes which later became so extensive that careful examination was needed to prove absence of voluntary control. The other rats showed flaccid paralysis passing into spastic in a few days. If function returned, motion, pain sensation and tactile sensation appeared usually in this order and all on the same side. When return began in one leg, a few days found function about normal. None of these rats developed leg edema, nor incontinent or distended bladders.

Since only one rat showed a return of function in a probably complete section of the cord, these may all be due to reëducation and vicarious functions of remaining elements. We believe actual regeneration has occurred, but must depend on histologic results not yet ready for final proof one way or another.

Young adult rats died in about eleven days after cutting or freezing the cord. Edema of the legs appears in eight days and the rats begin to eat them. Several showed at autopsy tremendous distention of the bladder with hydro-ureters and hydronephrosis. Care for this factor should make cord operations on older rats successful.

One rat, about four weeks old, following just subtotal section of the cord regained normal function in three weeks. The cord showed anatomic healing with a scar. Only the microscope can prove a true regeneration.

This work demonstrates: That intrauterine experimentation on rats is practicable; that transection of the embryonic spinal cord is followed by full function; that in new-born or older rats rapid return of function follows severe cord lesions, and probably this is due to a true regeneration.

The absence of permanent diabetes following pancreatectomy in the domestic fowl. T. Koppanyi, A. C. Ivy, A. L. Tatum and F. T. Jung.

We have removed the pancreas from the chicken by removing the duodenum and pancreas in toto, connecting the remaining portion of the small intestine to the stomach by an end-to end anastomosis.

We have had three chickens to survive the operation as long as 9, 30 and 40 days. The results are uniform. During the first 5 to 7 days glycosuria is present and the blood sugar runs as high as 9 to 13 mgm. per 1 cc. of blood.

After that period the glycosuria disappears and the blood sugar returns to normal, not exceeding 2 to 3.5 mgm. per 1 cc. The two chickens that lived 30 and 40 days died from inanition, probably due to disturbance of intestinal digestion because of lack of the external secretion of the pancreas.

Thus, the removal of the pancreas of the domestic fowl leads to a diabetes that endures only for a short time. It is apparent then that the internal secretion of the pancreas of this animal, if any, is either not specific, or can be formed by other organs.

Effect of adrenalin on the temperature of skeletal muscle after stopping the venous blood-flow through the liver and after stopping both venous and arterial blood-flow through the liver. M. W. CASKEY and E. J. HUMEL.

Adrenalin chloride was injected intravenously into six dogs, after ligation of the mesenteric arteries and all abdominal veins which might carry blood to the portal vein. An increase in the temperature of the skeletal muscle was noted. The average increase was about 0.12°C. The hepatic artery was then ligated and adrenalin was again injected intravenously. There was no observed rise in temperature in any case. In two cases blood pressure records were taken during the course of the experiments. A fall in blood pressure amounting to 60 mm. Hg occurred at the time of the stopping of the venous flow through the liver. When the arterial flow was stopped, there was a further fall in blood pressure amounting to 40 mm. Hg.

One of us (M. W. C.) has previously obtained data (not yet published) on twelve dogs which showed a fall in blood pressure amounting to 60 mm. Hg after ligation of the hepatic artery and the portal vein. In these twelve cases the preliminary mesenteric ligations were not done. The same data show that adrenalin causes an increase in muscle temperature if injected before ligation of the hepatic artery and the portal vein and no increase, if injected after ligation of these vessels. These data suggest that the liver is an essential factor in the increase of muscle temperature following intravenous injection of adrenalin.

It seems clear that the above results are not obtained as a result of some circulatory disturbance, surgical shock, etc., since these factors were just as marked following the cessation of venous flow through the liver as following the cessation of both venous and arterial flow through the liver.

Smooth muscle reactions to guanidine and liver extract. O. O. STOLAND.

It has been found that guanidine salts cause a sustained rise in blood pressure in animals under ether anesthesia. This guanidine hypertension can be reduced by certain liver extracts. The action of both substances seems to be to a certain extent if not entirely peripheral.

The experiments here reported were carried out to determine whether these substances influence other smooth muscle than that of the vascular system. Strips of the upper jejunum of rabbit's intestine and uterine horns of virgin guinea pigs and rats were used in vitro in an oxygenated Tyrode's solution maintained at body temperature. The liver extract used was prepared by Eli Lilly & Company and was obtained from Dr. Ralph H. Major who had shown that it would reduce the hypertension of patients as well as experimental animals.

In every case tried one cubic centimeter or more of 1 per cent solution of guanidine carbonate added to the Tyrode's solution in which the tissue

was suspended produced an increase in tone in rabbits, intestinal strips and uterine horns of virgin guinea pigs and rats. This hypertonicity was always reduced upon addition of one cubic centimeter or more of liver extract. In some cases the tonicity was reduced to the normal level or less. The addition of liver extract to untreated preparations produced only a slight drop in tone. When hypertonicity was developed in uterine muscle by treatment with pituitary extract or barium chloride the addition of liver extract produced very little if any reduction in the tone. The hypertonicity produced by guanidine in uterine muscle was never reduced by the addition of solutions of histamine, peptone or choline. The histamine and peptone solutions produce on the contrary a further increase in tone while the choline has no effect.

The above results indicate that the action of liver extract is not due to histamine, peptone or choline and support the possibility of another active substance being present which may be responsible for the depressor action of liver extracts. This substance does not seem to be specific for smooth muscle of the blood vessels but is a general depressant for smooth muscle especially when the muscle is in a state of hypertonicity from guanidine. Hypertonicity produced by other agents than guanidine is not readily overcome by the liver extract.

The skin and the kidneys as regulators of the water content of frogs. EDWARD F. ADOLPH.

Frogs, like other freshwater organisms, live in a medium with which they are not in osmotic equilibrium. Formerly it was believed that excessive water continually entered their bodies through the skin, and was eliminated at an equal rate by the activity of the kidneys. Recently it has become evident that water does not pass inward through isolated skin so rapidly as it passes outward through it, between the same solutions.

To measure the relative importance of the skin and the kidneys in the maintenance of a constant body volume, urine was caused to accumulate by ligating the cloacas, while the frogs were placed in various solutions. It was found that water was excreted through the kidneys equally fast in all hypotonic media. All the urines collected had osmolar concentrations less than one-fourth that of frog blood. The rate of water excretion among individuals which were in water was proportional to the body weight and not to the surface area. Urine formation was almost entirely inhibited for at least 48 hours while frogs were either immersed in isotonic or hypertonic solutions of NaCl or were kept out of water in atmospheres saturated with moisture.

The rate of urine formation was not only unrelated to area of body surface, but also was often independent of water intake. At high temperatures water excretion was augmented and body weight was lost; at low temperatures the reverse occurred. During recovery from desiccation urine was formed at the normal rate, even though the water required for recovery was greater than the amount in the urine. After the subcutaneous injection of hypotonic solutions the urine was not increased, and in a humid atmosphere the water from such injections was eliminated through the skin. The only injected solutions which required elimination by the kidneys were isotonic NaCl and Ringer's solutions.

Isolated frog skin when oriented normally caused water to pass through it into Ringer's solution only one-tenth as rapidly as it did when turned inside

out. The passage of water into frogs through their skins was therefore regulated so that sufficient water was available to the kidneys for the excretion of solutes in the form of hypotonic solutions, while the kidneys were saved the task of excreting any considerable amount of water arising by "leakage" into the body. The two facts, that no body weight was lost, and that no less urine was formed, in M/10 NaCl as compared with pure water, point to the conclusion that intake of water does not depend primarily upon differences between the concentration of medium and the concentration of body fluids.

A. On the independence of electrical and mechanical activity in the mammalian ventricle. B. On the effects of pH changes on conduction in the heart. CARL J. WIGGERS and M. G. BANUS (by invitation).

A. The relationship of the electrical to the mechanical activity of the mammalian heart was studied by comparing the incidence of surface negativity at various points of the ventricles with the rise of pressure within the ventricles. Such comparisons are considered valid according to the following considerations: 1, the elevation of intraventricular pressure begins with the contraction of the thick muscular interventricular septum; 2, the septum is negative far in advance of the appearance of surface negativity; consequently if electrical activity is an accompaniment of mechanical change, surface negativity should nowhere precede the rise of intraventricular pressure, but, in many places, should follow by reasonable intervals.

Results of experiments were presented which showed 1, that the surface negativity over the entire right ventricle constantly precedes the rise of right intraventricular pressure; 2, that surface negativity over the left apex generally precedes the rise of left intraventricular pressure; 3, that surface negativity over the anterior and more basal surfaces of the left ventricle generally occurs synchronously with, or very slightly after, the elevation of left intraventricular pressure. These intervals, however, are never as long as we may reasonably anticipate. The results of these experiments, therefore, form the conception that electrical variations develop in the ventricles before mechanical contraction begins.

B. The observations of Andrus and Carter (1922–24) and of Drury and Carter (1924) indicate that, in general, a decrease in the pH of the perfusion fluid by mineral acids, causes, in perfused hearts, a prolongation of conduction time.

In order to determine whether similar effects occur when CO_2 non-volatile organic and inorganic acids are added to the normal, whole blood in sufficient quantity to affect its pH, a series of experiments are being carried out in which the conduction times from auricle to various points on the auricular and ventricular surfaces are determined while the heart rate is kept constant by rhythmic, artificial stimulation of the right auricle.

pH changes are determined electrometrically, equilibration of blood samples with existing samples of alveolar CO_2 being carried out by the method described elsewhere by one of us (Banus).

The results so far obtained and analyzed, show that when the pH of the blood is decreased by slow infusion of N/5 HCl, the conduction time from the auricle to the ventricular surface increases (3 cases) and the conduction time to the left auricle is likewise prolonged (2 cases).

A decrease in pH of the whole blood produced by rebreathing CO_2

mixtures, likewise, causes a prolongation in conduction time (from auricle to ventricular surface, 10 cases; from right to left auricle, 5 out of 6 cases).

The effects of organic acids could not be sufficiently analyzed to warrant a report at the present time.

If corroborated by a larger number of experiments such results show that the effects of pH obtained in the perfused heart also apply to the heart in an intact circulation.

Method for equilibrating blood with frequently changing tensions of alveolar CO_2. M. GARCIA BANUS, introduced by CARL J. WIGGERS.

In electrometric determination of blood pH with Clark's or similar electrodes, it is necessary to know the CO_2 tension of the blood, so as to subject it to equivalent CO_2 tension in the electrode vessel. This is easily done when working with the blood of a subject with normal respiration, where the alveolar CO_2 tension is known and is practically constant. It is not so, however, in animal experiments where this tension is a variable (asphyxia, anoxemia, acidosis, etc.). In such cases, a quick way of determining the alveolar CO_2 tension, and also of supplying a mixture of H and CO_2 at the proper tension, is a great help in determining the corresponding changes in blood pH. For this purpose a simple method has been devised:—A special tracheal cannula with a narrow side tube is inserted into the trachea. Through this a catheter, preferably no. 10, French, is introduced beyond the branching of the trachea, and connected with a large deflated rubber bulb by means of a stopcock. The bulb is filled by drawing a small sample of air during each expiration. The tension of CO_2 in the sample is determined by means of Mariotte's colorimetric method.

A mixture of H and CO_2 is obtained by regulating the flow from two tanks by means of fine needle valves. Part of the mixture is bubbled through a tube containing Mariotte standard bicarbonate and indicator solution. The needle valves are regulated until the color in this tube matches exactly that obtained with the sample of alveolar air. The gas mixture, in which the CO_2 tension then differs less than 1 mm. from that of alveolar air, is run through the Clark electrode vessel used for pH determination of the arterial blood which is drawn at the same time as the alveolar air sample. The whole process takes less than five minutes. This method can also be used for obtaining mixtures of air and CO_2 at a definite CO_2 tension.

Successive samples from the same animal in double determinations of E. M. F. show differences less than 0.001 volt, corresponding with a pH value with an error of $\pm$ 0.01

When artificial respiration is employed continuous observations of alveolar air can be made. Toward the end of each expiration a sample of alveolar air is mechanically drawn from the catheter through the Mariotte solution. This is accomplished by interrupting the suction by a stopcock moved in connection with the artificial respiration machine, and arranged in such a way that it is only open a short time after the inflation of the lungs has ceased.

Electric capacity of animal tissues under normal and pathological conditions. Preliminary report. GEORGE W. CRILE, AMY F. ROWLAND (by invitation) and GEORGE H. CRILE (by invitation).

This report is a continuation of biophysical studies of variations in animal tissues under normal and pathological conditions. The apparatus

whereby the measurements have been made is briefly described, the formulae in accordance with which the calculations have been made as well as the apparatus being those devised by Hugo Fricke and reported by him in the Physical Review (November, 1925). These preliminary studies have indicated that variations in capacity of the central nervous system and of the liver follow the injection of an acid and of an alkali; that the capacity of the central nervous system is altered after hepatectomy; that the injection of ether into the tissues markedly decreases their capacity; and that the capacity of the liver increases with the age and size of the animal. These measurements indicate, although they have not as yet proven, that in common with electric conductivity and temperature, the capacity of a tissue is a constant which is susceptible to change under varying conditions.

On the functional significance of the double innervation of the gastrocnemius muscle of the frog. McKEEN CATTELL.

The gastrocnemius muscle of the frog is usually innervated by fibers which leave the cord in both the VIII and IX lumbar roots (classification of Langley and Orbeli). In thirty frogs one or the other of these roots was cut close to its emergence from the cord, and the animals kept alive for observations on possible functional changes in the gastrocnemius muscle. In most cases there was great initial weakness or almost complete paralysis on the operated side from which, during the course of several months, there was gradual recovery and the function of the operated leg to all outward appearances became normal. In other frogs no disturbance whatever was apparent following the operation. In every case, however, when the animals were stimulated to prolonged activity, to produce fatigue, a definite weakness became manifest on the operated side. The weight of the muscle taken from three to six months after cutting one of its nerve roots did not differ from the control taken from the opposite side.

These observations indicate that under normal conditions the function of the gastrocnemius of the frog, although lacking in reserve power, may be carried on satisfactorily with only half its nerve supply intact, and they lend support to the conception of a pleurisegmental innervation of the individual muscle fibers.

Sympathetic responses to repeated epinephrin injections. McKEEN CATTELL.

It has been shown by a number of observers that successive equal injections of small doses of epinephrin produce a gradually increasing vascular response in pithed cats. The present experiments were undertaken to determine whether other sympathetic responses behave in a similar manner. Observations were made on the result of repeated injections of epinephrin on the magnitude of the effect on salivary secretion, dilatation of the pupil and retraction of the nictitating membrane in cats anesthetized with ethyl bicarbamate. Equal intravenous injections of a standard epinephrin solution were made every five to ten minutes during the course of the experiment, lasting several hours.

In the case of the salivary gland, in four out of five cats, the secretion of saliva from the given epinephrin injection gradually increased, reaching an average maximum four times greater than the initial secretion. This maximum was reached between the third and fifth hour after the first injection. One animal failed to show an increase.

Measurements of the dilatation of the pupil also showed a gradual increase in the effectiveness of the given epinephrin injections as the experiment proceeded. The average maximum increase in eight experiments was 68 per cent, which was reached between two and three hours after beginning the injections. In this group also there was one negative experiment. In the three cases in which the thyroid glands had been removed eight or more hours before the first epinephrin injection, similar increases in the response of the pupil occurred.

Observations on the retraction of the nictitating membrane gave no evidence of an increasing sensitiveness to the epinephrin injections, but on the contrary there was a gradual decrease in response as the experiment proceeded, probably due to fatigue.

On the supposed pluri-segmental innervation of muscle fibres. L. N. KATZ.[1]

The evidence which has been given, to prove or disprove the possibility of a pluri-segmental innervation of muscle fibres, is inconclusive. The quantitative results of Cattell and Stiles[2] in favor of a pluri-segmental innervation have not been confirmed by Fulton[3] and Samojloff.[4]

On repeating the experiments of Cattell and Stiles I found,[5] in agreement with them, that the sum of the tension developed in an isometric tetanus when the two components of the sciatic nerve are stimulated in succession, is greater than the tension produced when both are stimulated simultaneously. The average difference in this series was 41 per cent. Cattell and Stiles found an average difference of 70 per cent, and Samojloff and Wassiljewa, of 20 per cent.

The claim of Cattell and Stiles that this indicates that the majority of the muscle fibres in the frog's gastrocnemius are innervated from more than one spinal segment, is criticised. An alternative explanation is suggested, based on the fact that tension is a force with a directional quality, and for this reason the tension developed in the gastrocnemius is not the *sum*, but the *resultant* of the tensions of its individual fibres. The mean direction of the contracting fibres of any particular section of the muscle may be able to turn itself parallel to the long axis of the muscle as a whole, when one nerve root alone is stimulated, whereas, when both sections of the muscle are stimulated together, each may, to some extent, be pulling against the other, and the resultant force in the direction of the muscle as a whole may be less.

A crucial test was applied to the theory of pluri-segmental innervation, by repeating Cattell and Stiles' experiments, employing the heat production as a criterion of the number of muscle fibres activated. Heat has no directional quality and the deflections obtained should be simply additive provided that the contraction is isometric, and the initial tension and length constant. A special thermopile was constructed for the purpose.

It was found that the total heat developed on stimulating the two components of the sciatic nerve separately is the same, within experimental error, as the heat produced when the two components are stimulated simul-

[1] This investigation was made at the Physiological Institute University College, London, while holding a Fellowship of the National Research Council.

[2] Cattell, M. and P. Stiles. This Journal, 1924, lxix, 645.

[3] Fulton, J. F. Proc. Roy. Soc., 1925, B xcviii, 493.

[4] Samojloff, A. and W. Wassiljewa. Pflüger's Arch., 1925, ccx, 116.

[5] Katz, L. N. Proc. Roy Soc., 1925, B xcix, 1.

taneously. The difference on the average in 16 sets of observations on 16 preparations was 1 per cent. This indicates that there is no pluri-segmental innervation of frog's muscle fibres apart from possible infrequent and chance variations.

A study of parathyroidectomized rabbits. J. B. COLLIP.

As rabbits have been found to be relatively immune to injections of the parathyroid hormone, a study of the blood chemistry of these animals as influenced by parathyroidectomy has been undertaken. The effect of the extract on both normal and parathyroidectomized animals has also been studied in much greater detail.

Removal of the parathyroid glands from normal rabbits results in the early development of violent tetany and death. Death occurs within 30 hours, as a rule. The tetany manifested by these animals is of a most violent and unyielding type. Death follows in most cases within a few minutes after the tetanic state has become established. Intravenous injection of the parathyroid hormone at this time, if not too long delayed, has been followed by almost immediate relief from the condition. In other cases neither $CaCl_2$ solution nor the hormone have prevented early death.

Shortly after the removal of the glands the blood calcium starts to fall, the inorganic phosphorus meanwhile showing no appreciable change. Later the inorganic phosphorus rises and in the tetanic state is found to be greatly increased.

Injections of the hormone have delayed the precipitation of tetany for many days. In these cases the blood calcium and the inorganic phosphorus are maintained within normal limits. Death may occur however during the treatment and the only abnormal finding may be an increased blood phosphorus.

By the use of massive doses of the hormone the blood calcium has been raised in both normal and parathyroidectomized rabbits. The response of normal rabbits is not at all uniform however. Repeated doses of the extract in normal animals appear to have no effect on blood phosphorus. In this way the rabbit differs from the dog and cat and it is probable that for this reason overdosage phenomena are not produced.

Chronic meat intoxication in Eck's-fistula dogs. S. A. MATTHEWS.

Hahan, Massen, Nencki and Povlov and Salaskin (1892–1894) were the first to describe meat poisoning in dogs with Eck's fistula. Also they were the first to attempt the total extirpation of the liver in mammals. Further they observed that the ammonia content of the blood increased after Eck's fistula, and were tempted to credit the nervous symptoms induced by the ingestion of meat to the increase of ammonia.

Subsequent investigations have corroborated their findings in regard to the increase of ammonia in the blood, but not in sufficient quantities to account for the meat intoxication. It has been found that the increase of ammonia accompanies the intoxication, and may be a secondary factor, in that one of the first expressions of meat poisoning is gastric atonicity resulting in gastric stasis. The contents of the stomach undergo putrefaction. This is when the ammonia content of the blood is highest. Just as soon as the stomach is emptied by emesis ($\frac{1}{12}$ gr. apomorphin) followed by lavage the ammonia content of the blood drops and all symptoms of intoxication disappears within a few hours.

The primary cause of the gastric stasis remains unexplained.

The urine in Eck's fistula animals is generally alkali and there is a tendency for the magnesium-ammonium-phosphates to deposit in the pelvis of the kidneys and in the tubules. Often these deposits take on the form of friable stones. The kidneys tend to become nephritic.

The liver progressively undergoes degeneration and in from 10 to 12 months the parenchyma is almost completely gone. The glycogen content is much diminished and the blood becomes hypoglycemic. A diet of bread, milk and fresh uncooked flat bones (sternum and ribs) is supposed to keep Eck's fistula dogs in a good state of nutrition indefinitely.

Aside from the acute meat intoxication there may follow in from 10 to 12 months a chronic nervous disorder somewhat resembling multiple sclerosis, in which there was a progressive breaking down of the proprioceptive function of the central nervous system. In one dog closely followed the following syndrome was present. Incoördination in the hind legs on climbing steps, on taking and maintaining the defecation or urinating position. These symptoms eventually involved the neck, eyes and muscles of mastication. Intention tremors were marked. The exteroceptive function and all the somesthetic sensibilities remained unimpaired. In fact the animal exhibited all the classical symptoms of cerebellar extirpation.

Microscopic examination of the cord and brain showed the following lesions:

a. Cord and brain stem: degeneration of the anterior, posterior and lateral columns, principally confined to the periphery. The gray matter was not affected.

b. Medulla, mid-brain and basal ganglia: Perivascular infiltration, especially of the medulla and the corpus restiforme.

c. Cerebellum: Enormous infiltrations of the molecular layer with glia cells.

Kidney function in Necturus maculosus. H. L. WHITE and FRANCIS O. SCHMITT.

Sugar is present in protein-free fluid drawn from the glomerular capsules of spring Necturi. The blood sugar is about 60 mgm. per 100 cc. The bladder urine is sugar-free, as is fluid drawn from points one-half or even one-third the way down the proximal convoluted tubule. Fall and early winter animals usually have no blood sugar and the bladder urine, proximal tubule fluid and capsular fluid are all sugar-free. If the plasma sugar level is raised to between 40 and 50 mgm. per 100 cc. by a subcutaneous injection of 2 cc. of 1 per cent glucose the capsular fluid contains sugar while the bladder urine and proximal tubule fluid are sugar-free. The site of sugar reabsorption is thus localized in the proximal convoluted tubule.

There are 360 to 400 mgm. NaCl per 100 cc. of plasma in this form. Capsular fluid gives a heavy precipitate with $AgNO_3$ while fluid from the distal end of the proximal tubule shows only a light cloud. Further evidence that the proximal tubule is the principal site of reabsorption of osmotically active substances is afforded by the observation that dog red cells stained with methylene blue, which lake in 0.2 per cent NaCl, when injected into a glomerular capsule never lake in the capsular fluid but almost immediately disappear when they are washed down into the proximal tubule as filtration proceeds. Cells fixed in formalin are easily seen

throughout the entire tubular system. The site of chloride reabsorption is thus also seen to be in the proximal tubule. There are only 17 to 26 mgm. NaCl per 100 cc. of urine.

There are 4.5 to 5.5 mgm. inorganic P by Briggs' method per 100 cc. plasma. Capsular fluid has on every occasion but one failed to give a positive test for phosphate, although there were from 2 to 11 mgm. P per 100 cc. of urine. The test as applied will show phosphate in concentrations as low as between 1 and 2 mgm. P per 100 cc. It is seen that if there is any phosphate in capsular fluid it is present in lower concentration than in the plasma. The full significance of these reactions is not yet clear. We believe they afford evidence of a tubular secretion of phosphate.

Observations upon the action of the parathyroid hormone. N. B. TAYLOR.

Observations upon the effects of the extract may be discussed under the following heads:

1. Injection of extract into herbivorous animals. Though a dosage greatly in excess of that given to dogs was employed, rabbits, guinea pigs and mice were quite immune to the action of the extract. No definite symptoms arose.

2. The decline in the calcium curve as urgent symptoms ensue. While the calcium is rising in the blood the animal shows few definite symptoms. There may be some lassitude and depression but nothing more. As the more urgent symptoms ensue there is a coincident sharp decline in the calcium curve. The drop amounts to from 3 to 4 mgm. and is from 25 to 35 per cent of the previous rise.

3. The effect upon the titratable acidity and ammonia of the urine. An examination of the urine in from 18–24 hours after the first injection of the extract reveals a sharp rise in the titratable acidity of the urine. The ammonia excretion is also increased but not to the same extent, with the result that the percentage of ammonia to acid + ammonia falls. The excretion of acid in some cases is increased 300 per cent. This rise in the titratable acidity and fall in the acid to acid + ammonia ratio is closely coincident with the elevation of the blood calcium. During this time the animal shows no symptoms and appears in excellent spirits. After the first outpouring of acid the urine becomes progressively less acid until when urgent symptoms supervene and the terminal decline of the calcium curve occurs the acidity of the urine together with the ammonia excretion fall. The former in most cases completely disappears and the urine becomes alkaline to phenolphthalein. The ammonia excretion shows a much less pronounced fall so that the ammonia to acid plus ammonia ratio arises to as much in some cases as 100 per cent.

The interpretation of these results is difficult. A possible explanation for the terminal secretion of alkali is that the anoxemia produced by the increased viscosity of the blood and consequent slowing of the circulation produces increased breathing, a blowing off of CO_2 and a resultant alkalosis. Such has been shown to occur in rarefied air experiments[1] when a similar reduction in the titratable acidity and a rise in the ammonia to acid + ammonia ratio was observed. The mechanism by which the anoxemia is produced however is different in the two cases. It is difficult to see how the blood if it circulates less freely can have its CO_2 blown off. If the

[1] Haldane, J. S., A. M. Kellas and E. L. Kennaway. Journ. Physiol., 1919, liii, 181.

anoxemia is the result of inability of the blood to obtain oxygen on account of the slowing of the circulation how can it get rid of its CO_2 and produce alkalosis? Furthermore the initial outpouring of acid is left unexplained by this hypothesis.

Evidences bearing on the source of the testicular hormone. ROBERT M. OSLUND.

The theory that the interstitial cells secrete the testicular hormone rests largely upon the claims of Ancel and Bouin that after vasectomy, after castration with implantation of a testicular graft, during cryptorchidism and after x-raying sex activity persists in the absence of germinal epithelium but with interstitial cell hypertrophy. After repeating the experiments covering these points the writer is led to question the evidences offered.

Vasectomy has generally been complicated with cryptorchidism. In uncomplicated vasectomy the germinal epithelium remains normal and all stages of spermatogenesis are found.

In testicular grafts the germinal epithelium usually suffers some degeneration. Such tissue has been described as containing only Sertoli cells and interstitial cells. Illustrations found in the literature show that more than Sertoli cells are present. The tubules contain additional cells that are of germ cell lineage. Even spermatocytes have usually been found in graft tissue.

Cryptorchid testes are said to suffer complete atrophy of the germinal epithelium. This tissue is not, however, completely absent. This statement is supported by the fact that cryptorchid testes, at least those experimentally produced, regenerate when placed in the scrotum. It is also supported by microscopical observations of cryptorchid testes taken from man, dog, sheep, pig, bull, rat and guinea pig.

It has been claimed that x-rayed testes are devoid of germinal epithelium. Those results are not confirmed. After dosages of x-ray greater than rereported in the literature, cells that resemble spermatogonia are present and regeneration takes place. Briefly stated: other than Sertoli cells are present in the tubules of such testes.

Germ cells as well as interstitial cells are therefore always present in testicular material that produces a sex hormone. The evidences offered in support of the interstitial cell secretory theory have been overstated.

Evidences that indicate the source of the testicular hormone. ROBERT M. OSLUND.

Did Sertoli cells alone persist in tubules of testicular grafts, or cryptorchid testes and of x-rayed testes, the evidence that the interstitial cells secrete the testicular hormone might still be questioned as to its conclusiveness. For where only Sertoli cells appear to remain, regeneration of germinal epithelium takes place. Members of the germ cell line must remain and by their division restore the seminiferous epithelium.

In slides of regenerating testes, cell types have been found intergrading between Sertoli cells and spermatogonia. The "Sertoli" cells that remain in testicular grafts, in cryptorchid testes and in x-rayed testes are essentially germ cells. They are indifferent cells which form spermatogonia and are the Sertoli cells of the normal testis. These indifferent cells are germ cells which take part in producing the testicular hormone. When sex

differentiation becomes apparent in embryos the germ cells are undergoing division. At puberty when sex development increases the germ cells are again undergoing division while the interstitial cells are decreasing. When senescencee omes on and sex activity disappears the germ cells degenerate and the interstitial cells increase. In amphibia sex neutrality disappears when sex cords form. The secondary sex characteristics change with change in the type of germ cells. This condition is similiar in birds where removal of female germ cells with development of male cells brings out male plumage. In birds showing great seasonal changes the plumage of spring, the sex plumage, developes during the time that the germ cells are dividing while the plumage that developes in the fall during the time that the interstitial cells are increasing is dull and much alike in both sexes. The sex activity likewise, in general, parallels increase of germ cells. In some experiments sex activity has been augmented immediately following temporary degeneration. At such times the germ cells are rapidly dividing in the process of regeneration. These are but a few of the evidences which have led the writer to suggest that the testicular hormone is produced by the metabolic activity of the germ cells.

The optical registration of continuous changes in electrical conductivity of tissue. G. B. Ray and David Rapport.

This variation of the usual type of conductivity apparatus has been arranged in order that a photographic record of the sound changes in the telephone receiver could be taken and compared with the curve of activity of a tissue under consideration.

The essential portion of the apparatus is the usual type of Wheatstone bridge. The current supply to the bridge is an alternating current supplied by a vacuum tube oscillator, as described by Hunter and Patrick (Journ. Lab. Clin. Med., x, 343) amplified by a two-tube amplifier. By means of a capacity switch the frequency of the current can be changed from 250 to 1000 cycles. Minor variations in frequency are possible by variation of the inductance. The addition of the amplifier was found to be desirable in order that a sharp end point be obtained.

Further changes were made in that the out-put of the bridge passes through two more stages of amplification. The increased out-put then leads to a large adjustable telephone receiver which is connected to a heart sound segment capsule, as devised by Wiggers and Dean. Vibrations of the mirror of the capsule are recorded photographically.

The conductivity cell used in the experiments consisted of a large T tube, to the side branch of which is attached a cannula for the heart. Into one side of this is fused one electrode. One end of the cross arm of the T is attached to a pressure capsule in order that contractile changes may be recorded. The other end of this arm is connected to a stopcock which further leads to an out-flow valve and thence to a reservoir of saline. Another cannula, in turn connected to a stopcock and inflow valve, completes the perfusion system. The ventricle, the tissue used in these experiments, is connected to these cannulae and placed in a beaker of saline. The second electrode is placed in this beaker directly under the tissue.

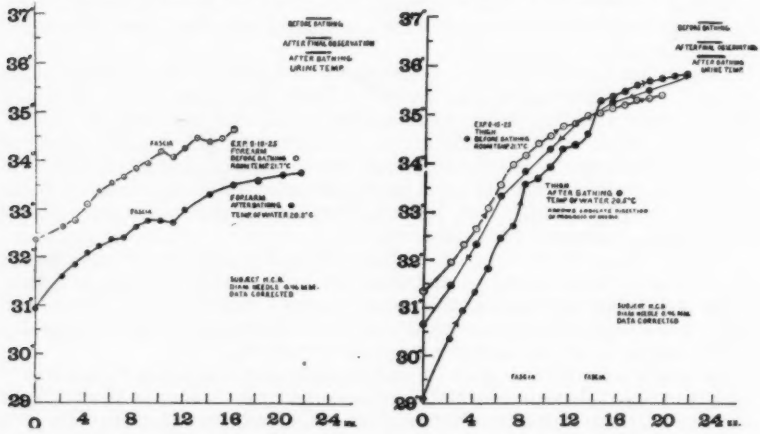
This apparatus has been found to be very sensitive, 1 mm. deviation from the zero on the slide wire causing a variation of 6 mm. on the sound record.

Changes in the electrical conductivity of the tortoise ventricle during activity. D. RAPPORT and G. B. RAY.

Electrical conductivity changes were measured in the tortoise ventricle during activity, simultaneously with a record of intraventricular pressure changes, by methods elsewhere described in these Proceedings. It was found that associated with contraction, and slightly preceding it, there is an increase of electrical conductivity, which reaches a maximum shortly before the greatest development of intraventricular pressure. Relaxation is accompanied by a decrease of electrical conductivity. Shortly before the onset of contraction there is a secondary increase in conductivity, which usually recedes before the beginning of the increase in conductivity associated with contraction. These changes are common to both isotonic and isometric contraction, though differing in degree. Possible sources of artefact, such as change in thickness of the tissue, movement of fluid past the electrodes, and shifting or twisting of the tissue, were controlled.

Temperature gradients in the tissues in the limbs in man. H. C. BAZETT and B. MCGLONE.

Isolated measurements of deep temperatures by thermocouples date from Becquerel and Breschet[1], with later measurements by Lefèvre[2] and similar measurements have been made with mercury thermometers by Zondek[3]. In the present study small needle thermocouples (modifications



of those described by Lefèvre) have been introduced to varying distances, allowing a more exact determination of the actual temperature gradients between the muscle and the surface. In general the needles have been introduced at right angles to the surface, either on the extensor aspect of the forearm or over the center of the sartorius muscle in the thigh, to depths of 20 to 28 mm. In other experiments needles have been introduced obliquely and x-rays taken to determine the exact depth. Surface tem-

[1] Becquerel et Breschet. Annal. des Sciences Nat. Zoöl., 1835 to 1837, 3, 4 and 7.
[2] Lefèvre. Arch. de physiol., 1898, 3.
[3] Zondek, B. Münch. med. Wochenschr., 1919, lxvi, 1315.

peratures have been taken by a small specially shaped wire junction of light weight (0.3 mm. diameter) allowing a considerable length of wire to be in contact with the skin and permitting temperatures to be taken at the point of entrance of the needle.

The relatively high thermal conductivity of the needles introduces a definite error, particularly if they are only introduced a short distance through an area where a large temperature gradient exists. This error can be greatly minimised by the use of oblique needles, and can also be estimated approximately, and the observed temperatures thus corrected. The error is less the smaller the needle, and needles as small as 0.35 mm. diameter, with a weight of 0.5 mgm. per millimeter length have been sometimes employed. Steel constantan junctions have been used throughout; a similar junction has been maintained at a known temperature in a thermos flask, the temperature difference being estimated from the galvanometer deflection after empirical standardization on each occasion.

This method confirmed the results of others as to the low temperatures existing in the muscles in the limbs under normal conditions. Types of the curves obtained under such conditions and also after a cold sea bath may be seen in the figure which represents a single experiment. Under normal conditions in this experiment the muscle temperature at a depth of 16.2 mm. in the arm was 2.25°C., in the thigh at 19.2 mm. 1.5°C. below the urine temperature, and this difference was of about the degree commonly observed. Immediately after bathing the gradient in the thigh was very much steeper, the subject shivered and complained of cold, and the muscle temperature (soon after shivering ceased) was above that previously recorded, although the urine temperature had fallen 0.68°. A little later temperatures observed in the arm were much below normal, but the curve had a normal form, and the subject no longer complained of cold. At this time the rectal temperature was rising, but the disappearance of the cold sensation can occur with very subnormal rectal temperatures, and without any measurable rise having occurred.

Similar variations in the slope of the curve were found under varying room temperatures.

Our thanks are due to the Ella Sachs Plotz Foundation for a grant towards the expenses of this research.

Blood changes and experimental cretinism, hyperthyroidism and myxedema.
MARGARETE M. KUNDE.

A. The following changes have been observed in the blood and tissues of rabbits with hypothyroidism.

1. There is marked secondary anemia, the hemoglobin may be 30 per cent to 40 per cent below normal with the red blood cell count reduced to $\frac{2}{3}$ normal.
2. The cholesterol content of the blood is markedly increased.
3. The calcium content may be slightly below normal or normal.

B. 1. Thyroid administration (either the desiccated by mouth or Kendall's thyroxin injected intravenously to hypothyroid rabbits produces a polycythemia, but the hemoglobin still remains below normal. The cholesterol content of the blood is decreased and the calcium slightly increased.
2. Water is extracted from the tissues, especially from the skin.
3. The cretin scales are high in cholesterol content.
4. The stimulating effects on the ovaries is apparent by the greatly increased number of Graafian follicles.

Importance of the liver with regard to the formation of bilirubin. FRANK C. MANN, JESSE L. BOLLMAN and CHARLES SHEARD.

Our previous work on the site of the formation and the source of bilirubin has proved that there is an extrahepatic site, that the bilirubin is made in the spleen and bone marrow, and that it is made from hemoglobin. While it was readily and conclusively shown that bilirubin is formed outside the liver, it is difficult to determine to what extent the liver is responsible for the formation of the pigment. Several series of experiments were performed. Those affording the most suggestive results consisted in studying the biliribuin content of the blood following removal of from 70 to 80 per cent of the liver of a normal dog, or from one in which an Eck fistula had been established previously. Following this procedure a moderate transitory bilirubinemia developed and usually persisted for from two to five days. The bilirubinemia appeared to depend on failure of the liver to excrete the bilirubin which had been formed elsewhere. The rate as well as the amount of bilirubin formation was found to depend on several factors. The results of all our experiments have tended to minimize the importance of the liver as a site of the formation of bilirubin.

The effect of colectomy on the incidence of parathyroid tetany. E. OLDBERG and A. C. IVY.

Because of the observations of Dragstedt and of Luckhardt, which tend to show that the cause of tetany following parathyroidectomy is absorption of toxic materials from the intestine, we have attempted to ascertain the effect of colectomy on this phenomenon.

We have obtained results on seven dogs from which the thyroid-parathyroids have been removed a week or two after colectomy.

Four of our animals have lived three days, three days, seven days, and twenty-three days respectively, without showing any evidence of tetany following removal of the parathyroids.

Three of our animals have shown mild tetany which appeared on the third, sixth, and tenth days respectively, and which recurred at intervals of from two to three days. This tetany was easily relieved by washing out the lower ileum with warm isotonic sodium chloride solution.

Two dogs have lived over four weeks without colons and without any apparent ill results. Our experiments show that colectomy much delays the onset of, and considerably moderates, the symptoms of tetany following the removal of the thyroid-parathyroid gland substance.

Demonstration of a dog with a transplanted gastric pouch and of a dog with a pancreatic transplant. A. C. IVY and J. I. FARRELL.

A dog that has had a pouch of the stomach transplanted into the mammary gland now for about one year was demonstrated. This pouch is as mobile as the mammary gland and secretes an acid juice following a meal, gastrin and histamine. A roentgenogram of a pneumoperitoneum done on this dog was demonstrated, showing the absence of adhesions or connections between the transplant and abdominal viscera.

A dog that has a pancreatic transplant in the mammary gland was demonstrated. This animal was fed and secreted throughout the course of the demonstration. A roentgenogram of a pneumoperitoneum done on this dog was demonstrated, showing the absence of adhesions or connections between the transplant and abdominal viscera.

A preliminary report on a "therapy" for peritonitis. H. M. KELBY and A. C. IVY.

On observing dogs that had a peritonitis caused by bile and urine drainage into the peritoneal cavity, it occurred to us that intestinal obstruction, due to both spastic and paralytic ileus, might play a rôle in death from peritonitis due to any cause.

On this basis, dogs with an experimental peritonitis were treated by a method devised to relieve intestinal obstruction. A jejunostomy was made, through which a tube was passed up into the duodenum and down into the ileum. These tubes served as drainage tubes and as a means of providing fluid and nourishment. From two to five hundred cubic centimeters of Ringer's solution or predigested milk were injected into the intestine every four hours. Peritonitis was produced, either by making an open loop of fifteen inches of the upper ileum, or, in some cases by injecting feces. The results of experiments in which peritonitis was produced by injection of feces were unsatisfactory because of the great variability in the potency of different feces suspensions.

Fifteen control dogs with an "open loop" died in from three to five days. Only one control dog survived as long as two weeks, but he was kept on a therapy of intravenous Ringer's solution, which, however, failed in other cases. Two out of four "open loop" dogs that received the treatment outlined above for the prevention of obstruction and the administration of fluid survived over two weeks, dying of causes other than peritonitis. One is now alive and healthy at three weeks, and the other died on the fourteenth day. The operated controls for this series died on the second and third day following the production of the "open loop." These observations, although very encouraging, cannot be considered as conclusive until further data of a similar nature have been collected.

Studies on the motility of the transplanted gastric pouch. J. I. FARRELL and A. C. IVY.

We have studied the motility of the gastric pouch transplanted into the mammary gland for the purpose of studying the secretion of such a transplant.

By following the motility of the stomach proper simultaneously with the motility of the transplanted pouch, we have made the following observations:

a. When the stomach proper contains food and does not show hunger motility, the transplant likewise shows no motility.

b. Just prior to a "hunger period" in the stomach, the transplant manifests an increase in tone and ten to twenty second contractions that occur without time relation to the contractions in the stomach. These contractions increase to a maximum along with the increase of those in the stomach.

c. When the stomach proper enters a "rest period," the contractions of the transplanted pouch decrease in amplitude and the tone gradually diminishes until a period of rest occurs.

d. Food by stomach tube inhibits the motility of the transplant in from five to ten minutes.

e. Fat by stomach tube inhibits the motility within five minutes usually.

f. Glucose intravenously in relatively large amounts also inhibits in about five minutes.

These observations prove that the hunger contractions in the stomach are caused by a humoral mechanism.

On the prevention of parathyroid tetany by the oral administration of magnesium chloride. ARNO B. LUCKHARDT, R. O. WAUD and LONDUS BRANNON.

Twenty grams of magnesium chloride were administered daily to 8–16 kgm. dogs by stomach tube in divided doses of from 5 to 10 grams in 250 cc. of water following thyroparathyroidectomy in order to determine the effect of magnesium chloride in preventing tetany and in order to study the blood serum calcium level while the animals were under the influence of this agent.

Results: In those animals which retained the magnesium chloride we observed: 1. A progressive fall of the blood calcium from the initial level of 10 to 12 mgm. to a figure in many instances appreciably below 7 mgm. without any signs of the neuro-muscular phenomena so characteristic of parathyroid tetany. In one animal the average blood calcium from the 16th to 22nd day was 5 mgm. Two other dogs were maintained free from tetany with the blood serum calcium as low as 4 mgm.—the average daily blood Ca for a period of 6 days being 5.6 mgm. in each case (blood calcium before operation was 11.8 and 13.1 mgm. respectively).

2. We have repeatedly observed tetany in animals with a blood calcium *higher* than it was on some preceding or succeeding days when the animals were free from any apparent symptom of tetany (e.g., 6.1 mgm. without tetany versus 7.51 mgm. with tetany).

3. In several of our experiments there is an indication that the administration of magnesium chloride leads to some slight mobilization of body calcium.

4. It should not be inferred, however, that these animals, kept alive by the oral administration of magnesium chloride, were as normal as those kept alive by the oral administration of calcium salts. Contrary to the prevalent belief that magnesium chloride, when administered by mouth, produces no systemic effect other than catharsis, we found that it has the following pharmacologic action:

Initial drooling, marked catharsis usually, lack of appetite leading to complete anorexia, polydypsia, somnolence, flaccidity of the skeletal musculature, incoördinate staggering gait, disappearance of the patellar jerks, blunting of cutaneous sensibility, slowing of respiration to 8 per minute, in short, a form of anesthesia as noted by others following the *subcutaneous* administration of a magnesium salt. Without definite evidence in its support we venture the opinion that the preservation of life of parathyroidectomized animals by means of the oral administration of magnesium chloride is due essentially to a depression of the irritability of the central nervous system preventing thereby the rapidly fatal convulsive seizures which would otherwise occur.

A method for studying the function of the sympathetic innervation of the small intestine, with preliminary results. J. E. THOMAS.

A study of the sympathetic innervation of the small intestine has been undertaken by means of excised preparations. These consist of two or three inches of excised intestine, with the mesentery still attached, functioning in oxygenated Locke's solution at 38 degrees centigrade. Elec-

trical stimulation in the region of a mesenteric blood vessel excites the vagus and sympathetic fibers to the intestine. In the preparations studied, the vagus fibers had been eliminated by section of the vagus trunks in the thorax five weeks previously. By the use of excised material vasomotor and adrenal effects that complicate splanchnic stimulation in the experimental animal, are eliminated.

Preliminary results have been obtained on the intestine of two cats. The effects of stimulating the sympathetic nerve fibers consist of primary inhibition followed by augmentation affecting both tonus and rhythmic contractions. The secondary motor effect is approximately equal in extent and duration to the primary inhibitory effect. Although both longitudinal and circular muscles from various levels of jejunum and ileum have been studied the results have been qualitatively similar in all preparations. Likewise changing the strength or duration of the stimulus has not altered the general character of the result.

The inhibitory effect is probably due to sympathetic fibers having an inhibitory function. The motor effect may be due to 1, sympathetic fibers having a motor function, or 2, changes in the muscle induced by the condition of inhibition, or 3, the presence of vagus fibers that escaped section. The third possibility would be probable only in case accepted views of the course of the intestinal vagus fibers should prove to be erroneous.

The seasonal variation in the catalase content of warm blooded animals and evergreen trees. W. E. BURGE.

Catalase determinations were made weekly throughout the period of a year of the needles of several species of pines growing in this vicinity. It was found that the catalase increased as the weather became warmer, passing from winter to spring and summer, and was highest in the summer when the weather was hottest and decreased when the weather became colder, passing from summer to fall and winter, and was lowest in the winter when the weather was coldest.

Similarly, catalase determinations were made of the blood of white rabbits kept in an open court and hence exposed to the same temperature as were the evergreen trees, and it was found that as the weather grew colder, passing from summer to fall and winter, the catalase increased and was highest in the winter when the weather was coldest and decreased when the weather became warmer, passing from winter to spring and summer, and was lowest when the weather was hottest.

It is known that a fall in temperature decreases the oxidative processes in plants and increases it in warm blooded animals and that the reverse is true with a rise in temperature. By comparing the above results, it may be seen that a fall and rise in temperature has the same effect on catalase that it has on the oxidative processes.

The effect of anesthetics on the sugar metabolism and catalase content of unicellular organisms. W. E. BURGE.

The anesthetics used were chloroform, ether, nitrous oxide, and ethylene. Paramecia caudata were the unicellular organisms used.

A large quantity of the organisms was collected and divided into two equal portions, each of which was introduced into 200 cc. of a 0.2 per cent dextrose solution. One of the portions was used to try out the effect of

the anesthetics, while the other served as a control. A mixture of the anesthetic and oxygen in gaseous form was bubbled through the liquid containing the organisms at the rate of a bubble every few seconds. Catalase and sugar determinations were made at intervals.

It was found that chloroform and ether produced a marked decrease in the catalase content and sugar metabolism of the organisms, while the nitrous oxide and ethylene produced very little decrease in either the catalase or the sugar metabolism.

The effect of insulin, thyroxin and temperature on the sugar metabolism of unicellular organisms. W. E. BURGE.

A large quantity of the unicellular organisms, paramecia caudata, were collected and divided into two equal portions, each of which was introduced into 200 cc. of a 0.2 per cent dextrose solution. To one portion a tube of U-20 insulin was added. At eight hour intervals sugar determinations were made according to Benedict's method and it was found that the organisms to which the insulin was added metabolized the sugar much more rapidly than the controls.

In a similar manner a large quantity of the organisms was collected and divided into two equal portions. One portion was kept at ordinary room temperature, 22°C., while the other was kept at 2°C. It was found that the low temperature decreased the metabolism of sugar to a marked degree.

The effect of varying amounts of thyroxin was also tried and it was found that this material had little or no effect on the sugar metabolism.

The effect of x-ray on the pancreas. N. F. FISHER, J. T. GROOT and A. BACHEM.

By removing the head and body of the pancreas it is possible to administer known amounts of x-ray to the gland without at the same time affecting other tissues. The application of three erythema doses to the pancreas causes marked fibrosis of the pancreas and diabetes. From this condition the pancreas can recover by proliferating new tissue from the periphery of the gland. Four or five erythema doses applied to the pancreas cause complete disappearance of the gland. Polyuria without glycosuria developed in one dog on the third day following the exposure to four erythema doses. Glycosuria occurred in one case to the extent of 8.8 per cent without polyuria. One dog lived 57 days with complete intolerance to carbohydrates, which is 15 days longer than the maximum length of life reported for a pancreatectomized dog not receiving insulin.

A chemical test for sex. DEWEY G. STEELE and AGNES L. ZEIMET.

Probably the most significant contribution to the study of sex made in recent years is the chemical test for sex devised by a Russian physician, E. O. Manoiloff. This test may be made on the blood of animals or the alcoholic extracts of certain plants. A color difference is the basis for reading the test.

Several workers have confirmed this test with a large number of animals. In all these studies only about 70 to 80 per cent accurate tests have been secured. We find the Manoiloff test too complicated for general use. For this reason we have shortened the process with as good results as before. More than 150 animals, including cattle, pigeons and fowls have been tested by either the original or the modified methods.

The test may be applied not only to blood but also to the pulp of growing feathers. The striking thing about the feather test is that the color change is the reverse of that given by the blood. Young chicks (1 to 12 days old) and pigeon squabs (3 to 4 weeks old) have been used for this test. It has not yet been possible to try this test on growing feathers from mature birds.

Gurewitsch has used aesculin in addition to the Manoiloff reagents in testing maternal (human) blood for the sex of the fetus.

The Manoiloff test is based on oxidative reactions. Manoiloff and Gurewitsch believed that qualitative differences are concerned. However the results obtained in our chemical studied indicate that quantitative rather than qualitative differences are involved in the reaction. Quantitative studies are now in progress on the reducing substances of male and female blood. These studies are to be reported more fully later.

The action of adrenalin and acetyl-cholin on the coronary arteries of the rabbit. FRED M. SMITH, G. H. MILLER and V. C. GRABER.

The present investigation is a preliminary study of the action of the sympathetic and vagus nerves on the coronary arteries of the rabbit. The effect of adrenalin and of acetylcholin on the coronary flow of the perfused heart of the rabbit was studied because of the action which these drugs are said to have on the extrinsic cardiac nerves.

The heart was perfused at a pressure of 55 mm. Hg with a Locke Ringer solution saturated with oxygen. The perfusate was kept at a constant temperature of 38°C. The rate of coronary flow was recorded by the tipping bucket method. A uniform cardiac rate was maintained by rhythmically induced break shocks in some experiments. The rhythmical stimulation was produced by an oscillating contact maker, employing the principle of the tuning fork which was connected with the ordinary induction coil.

Adrenalin was introduced into the perfusate by injecting it with a hypodermic syringe directly into the tubing at its attachment to the aortic cannula. The concentration of adrenalin used was 1–10,000,000, 0.2 cc. of this being injected at a rate which would produce a dilution of approximately 1–200,000,000 in the coronary arteries. The above low concentration was employed to avoid the complicating effects produced by the extreme cardiac stimulation which results from the higher concentrations that are ordinarily introduced. Adrenalin in this dilution produced a definite decrease in the rate of coronary flow. The decrease in the rate of perfusion varied from 12 to 22.5 per cent. The diminished perfusion rate began immediately after the introduction of the drug, and persisted up to the point where the cardiac stimulating action of the drug was at its height. At this point the rate of coronary flow was slightly augmented for a short time. Even with these low concentrations of adrenalin there was a very distinct stimulating effect on the heart, as indicated by the acceleration in heart rate and increase in amplitude of contraction.

Acetyl-cholin was introduced into the coronary arteries in dilutions varying from 1–100,000 to 1–200,000. The drug in these concentrations greatly reduced the heart rate, and in some instances produced cardiac standstill. Coincident with the reduction in the rate, the amplitude of the cardiac contraction was diminished. With the onset of the above changes in the heart, the perfusion rate was greatly augmented. In some instances the increase was 180 per cent. The slowing of the cardiac rate and the

depression in amplitude of contraction were transient. The administration of atropin sulphate in a dilution of 1–20,000 eliminated the effects of subsequent doses of acetyl-cholin on heart rate, and in a large measure the reduction in the amplitude of contraction. The atropin, furthermore, eliminated the action of acetyl-cholin on the perfusion rate except for very transient slight increase during the period in which there was some depression in amplitude of contraction.

In those experiments in which the cardiac rate was controlled by rhythmical stimulation, thus eliminating, in a large measure, the depression in the amplitude of contraction, the dilating action of acetyl-cholin on the coronary vessels was still observed.

Physical exercise and auditory acuity. GRACE A. GOLDSMITH, LENORE S. LUENZMAN, LOUISE SAMMONS and BEULAH W. ZIMMERMAN.

Interest in this topic was aroused by the work of A. F. Stanley Kent (1916).

In our experiments the source of sound was a watch mounted on a carriage which could be slid back and forth on rails.

In confirmation of Kent a daily and weekly variation in acuity was shown in which the acuity falls at night and at the end of the week. This is in striking contrast with the diurnal variations in reaction time which usually show an improvement at night as indicated in the unpublished work of G. Fellows and C. B. Williams (1919) in the case of female students of physical education and as sometimes found among operatives by Kent (l. c.).

The effect of physical exercise depends upon the amount of the exercise and the condition of the subject. In the fresh or only moderately fatigued subject moderate exercise increases the acuity while in subjects who are already much fatigued even moderate exercise decreases the acuity. Severe exercise always decreases the acuity. Sometimes a fall in the acuity gives warning to the observer of the onset of an indisposition on the part of the the subject some hours before the subject becomes aware that anything untoward is happening to himself. Mental work also produces decrease of auditory acuity.

Martin's strength test in normal children. KATHERINE E. BURTNESS, MARION J. COREY, EDITH MAPLESDEN and M. HELENA OLESEN.

When E. G. Martin devised his strength test to meet the emergency of an epidemic of poliomyelitis anterior, it was at the same time necessary to secure a normal standard with which his prospective patients might be compared. The standard secured by him (1918) was based largely upon the study of children in the orthopedic hospital. Statistics from these children were very cautiously and carefully handled by him and proved to be at least temporarily adequate.

It seemed to us, however, that more normal material should be examined and additional statistics gathered if, as appears not improbable, this test is to become a permanent instrument of physiologic anthropometry.

We therefore measured (long test) 120 boys and 60 girls from the Madison schools and present our averages in the accompanying table. The age groups contain equal numbers of subjects, viz., 24 boys and 16 girls. The distribution of strength among the various muscle groups will appear in the complete report.

	AGE									
	10 years		11 years		12 years		13 years		14 years	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Weight	72.40	65.76	76.10	68.40	81.70	84.14	96.90	97.89	106.30	103.60
Strength	1600	1407	1700	1552	1797	1671	2017	1685	2162	1856
S/W	22.09	21.39	22.33	22.69	21.99	19.85	20.81	17.23	20.33	17.91
Height	53.80	53.20	55.79	54.84	57.96	58.20	57.98	60.82	62.40	62.56
S/H	26.02	26.40	30.40	28.30	31.00	28.70	34.70	27.70	34.40	29.76

Schneider's and Crampton's tests in athletic and unathletic girls. MARIE K. DAMEZ, DOROTHY MATHIS and MARGARET MURRAY.

The cardiovascular ratings of E. C. Schneider (1920) and the ptosis percentage of C. W. Crampton (1913) were obtained in a study of 200 schoolgirls, half of whom were engaged in athletics while the other half were not. The diastolic pressure was also determined.

The data thus secured seem to us to indicate that these tests (especially Schneider's) will prove helpful in determining the degree of physical efficiency present in a given individual.

Among the twenty-two results or conclusions arrived at in the study of these data are the following:

In athletic girls the ratings by Schneider's and Crampton's methods are higher than in the unathletic. In the former the range of the ratings with both of these methods is smaller—the subjects are more nearly standardized. There is no acceptable degree of correlation between the results of these two tests. Girls with a Schneider grade below 6 or a Crampton percentage of less than 70 should be observed with especial care by those who direct their physical activities. In athletic girls the various factors of the Schneider test have the superiority over those of the unathletic in the order A, D, B = E, C, F, and it would therefore seem that the factors A and D are of greatest importance as criteria of physical efficiency while C and F are of least importance.

The accuracy of quantitative determinations of basic ions. MARY WHELAN.

The methods commonly used for the quantitative determination of the inorganic basic ions were tested carefully with accurately prepared known solutions and on urines. This was done in order to check the individual methods as well as to determine the accuracy of Fiske's method for total fixed base when applied to physiological fluids such as the urine. The percentages of accuracy of the methods when determinations were made on the solution of known composition were compared with those obtained when a known solution was added to urine and the percentages of recovery calculated. It has been found that the percentage of recovery of each substance falls within the accuracy of the method. A positive error of about 3 to 5 per cent was obtained for each of Kramer and Tisdal's methods for the determination of sodium potassium and calcium. The magnesium method of Kramer and Tisdal and Briggs gives a positive error of about 15 per cent in concentrations equal to those in blood. A negative error of less than 1 per cent was obtained for Fiske's fixed base method as an average of about twenty determinations. The sum of these four estimations in urine was quite comparable to results obtained by Fiske's total base method.

A preliminary note on the blood picture in dogs following experimental atrophic gastritis induced by sodium fluoride. C. D. LEAKE and G. RITCHIE.

That changes in the blood picture might follow experimental atrophic gastritis was suggested by the clinical association of achlorhydria with pernicious anemia, and the clinical and experimental observation that gastrectomy is followed by changes in the blood picture simulating those found in pernicious anemia. Twenty-five cubic centimeters of a 5 per cent solution of sodium fluoride were administered by stomach tube once or twice weekly to dogs after atropinization, and were followed by 50 cc. of 10 per cent calcium carbonate. The dogs gradually lost weight although appetite seemed well maintained. The diet included meat, milk and dog biscuits. The animals became listless, irritable and often had diarrhea. The gastric acidity was reduced, in some cases to zero. There were fluctuations in the erythrocyte count succeeded by a decline amounting to over two million cells after ten weeks. The oxygen capacity of the blood diminished meanwhile five or more volumes per cent. Normoblasts and anisocytosis were observed. When the administration of sodium fluoride was discontinued, gradual recovery ensued. Control dogs receiving only calcium carbonate developed similar but less marked changes in the blood picture. In animals killed after ten weeks' administration of sodium fluoride, the histological changes found were slight atrophic gastritis, particularly in the fundic region, and a moderate extension of the red bone marrow. There was also an abnormal deposition of iron pigment in the spleen and red bone marrow. Unfortunately no studies were made of the bacteriology of the intestinal contents.

Whatever may be the organisms or processes leading to the clinically irreversible end-picture of pernicious anemia, may it not be worth while to include a gastric analysis more commonly in medical examinations, and if subnormal acidity is found, to institute hydrochloric acid therapy, in the effort to reduce the incidence of the disease?

The effect of hysterectomy on the estrus cycle of the white rat. E. P. DURRANT.

Twelve rats were hysterectomized when from 108 to 126 days old and the results noted by daily examination of the vaginal smears. The smears revealed a complete cessation of the cyclic vaginal changes for thirteen to thirty-three days after which they were completely restored. In a second group of fourteen rats with a somewhat wider range in age the effects of hysterectomy were observed by means of the revolving activity cage. Daily observations by this method showed no change in the characteristic cyclic activity, although there was a decrease in total activity for a few days immediately following the extirpation of the uteri. It is believed that the temporary cessation of the estrual changes in the vaginal mucosa was due to operative interference with the circulation of the vagina and that the uterus has no significant influence on the periodic activity of the ovary.

Where Pignet's index fails. JOHN F. MOON, HELEN PARKER, MABEL E. RUGEN and CARL E. SIBILSKI.

In 1900 a French army officer, Major Pignet, published a series of articles in the *Archives Medicales d'Anger* in which he discusses a new index of efficiency of his own devising. The index is *height*—(*chest girth* + *weight*) and the lower the value the more efficient the individual according to Pignet.

Our results show 1, that the relations which Pignet finds between these factors (height, etc.) in French recruits do not hold for 125 Wisconsin college women and still less do they hold for 150 Wisconsin college men. 2, That the correlation between this index and the strength in 150 college men is practically nil, and 3, that its correlation in 125 college women with the gymnastic efficiency used at Wisconsin is of no significance whatever.

Pignet's figures also show that the morbidity is less among soldiers with small indices. Whether this be true in the case of American college students is now the subject of investigation.

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